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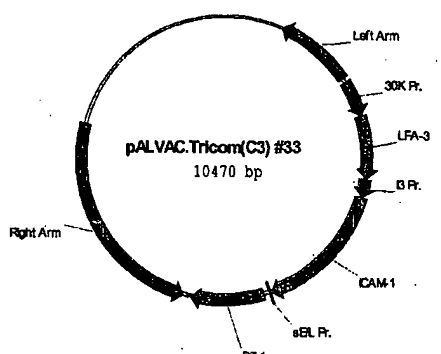
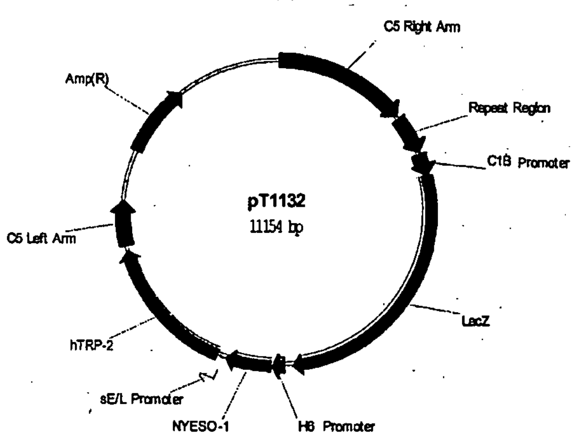
(54) Title: MULTI-ANTIGEN VECTORS FOR MELANOMA





(57) Abstract: The present invention relates to peptides, polypeptides, and nucleic acids and the use of the peptide, polypeptide or nucleic acid in preventing and / or treating cancer. In particular, the invention relates to peptides and nucleic acid sequences encoding such peptides for use in diagnosing, treating, or preventing melanoma.

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Multi-Antigen Vectors for Melanoma

FIELD OF THE INVENTION

The present invention relates to multi-antigen vectors for use in preventing and / or treating cancer. In particular, the invention relates to multi-antigen vectors for use in treating and/or preventing melanoma.

BACKGROUND OF THE INVENTION

There has been tremendous increase in last few years in the development of cancer vaccines with tumour-associated antigens (TAAs) due to the great advances in identification of molecules based on the expression profiling on primary tumours and normal cells with the help of several techniques such as high density microarray, SEREX, immunohistochemistry (IHC), RT-PCR, in-situ hybridization (ISH) and laser capture microscopy (Rosenberg, Immunity, 1999; Sgroi et al, 1999, Schena et al, 1995, Offringa et al, 2000). The TAAs are antigens expressed or over-expressed by tumour cells and could be specific to one or several tumours for example CEA antigen is expressed in colorectal, breast and lung cancers. Sgroi et al (1999) identified several genes differentially expressed in invasive and metastatic carcinoma cells with combined use of laser capture microdissection and cDNA microarrays. Several delivery systems like DNA or viruses could be used for therapeutic vaccination against human cancers (Bonnet et al, 2000) and can elicit immune responses and also break immune tolerance against TAAs. Tumour cells can be rendered more immunogenic by inserting transgenes encoding T cell co-stimulatory molecules such as B7.1 or cytokines such as IFN-γ, IL2, or GM-CSF, among others. Co-expression of a TAA and a cytokine or a co-stimulatory molecule can develop effective therapeutic vaccine (Hodge et al, 95, Bronte et al, 1995, Chamberlain et al, 1996).

There is a need in the art for reagents and methodologies useful in stimulating an immune response to prevent or treat cancers. The present invention provides such reagents and methodologies that overcome many of the difficulties encountered by others in attempting to treat cancer.

SUMMARY OF THE INVENTION

The present invention provides multi-antigen vectors for administration to a patient to prevent and / or treat cancer. In particular, the multi-antigen vector encodes one or more tumor antigens ("TA"). The multi-antigen vector may also encode an immune stimulator such as a co-stimulatory molecule and/or be administered with an adjuvant.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic of plasmids pALVAC.Tricom(#33) and pT1132.

Figure 2. DNA sequence of plasmid pALVAC.Tricom(#33).

Figure 3. DNA sequence of plasmid pT1132.

Figure 4. Schematic of plasmid pT3217.

Figure 5. DNA sequence of plasmid pT3217.

Figure 6. Amino acid sequences of exemplary NY-ESO-1, TRP-2, gp100, gp100M, MART-1, MAGE-1, MAGE-3, B7.1, LFA-3, and ICAM-1 proteins.

DETAILED DESCRIPTION

The present invention provides reagents and methodologies useful for treating and / or preventing cancer. All references cited within this application are incorporated by reference.

In one embodiment, the present invention relates to the induction or enhancement of an immune response against one or more tumor antigens ("TA") to prevent and / or treat cancer. In certain embodiments, one or more TAs may be combined. In preferred embodiments, the immune response results from expression of a TA in a host cell following administration of a nucleic acid vector encoding the tumor antigen or the tumor antigen itself in the form of a peptide or polypeptide, for example.

As used herein, an "antigen" is a molecule (such as a polypeptide) or a portion thereof that produces an immune response in a host to whom the antigen has been administered. The immune response may include the production of antibodies that bind to at least one epitope of the antigen and / or the generation of a cellular immune response against cells expressing an epitope of the antigen. The response may be an enhancement of a current immune response by, for example, causing increased antibody production, production of antibodies with increased affinity for the antigen, or an increase in the cellular immune response (i.e., increased number or activity

of immunoreactive T cells). An antigen that produces an immune response may alternatively be referred to as being immunogenic or as an immunogen. In describing the present invention, a TA may be referred to as an "immunogenic target". The present invention provide expression vectors for expressing in a host one or more immunogenic targets.

The term TA includes both tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs), where a cancerous cell is the source of the antigen. A TAA is an antigen that is expressed on the surface of a tumor cell in higher amounts than is observed on normal cells or an antigen that is expressed on normal cells during fetal development. A TSA is an antigen that is unique to tumor cells and is not expressed on normal cells. TA further includes TAAs or TSAs, antigenic fragments thereof, and modified versions that retain their antigenicity.

TAs are typically classified into five categories according to their expression pattern, function, or genetic origin: cancer-testis (CT) antigens (i.e., MAGE, NY-ESO-1); melanocyte differentiation antigens (i.e., Melan A/MART-1, tyrosinase, gp100); mutational antigens (i.e., MUM-1, p53, CDK-4); overexpressed 'self' antigens (i.e., HER-2/neu, p53); and, viral antigens (i.e., HPV, EBV). For the purposes of practicing the present invention, a suitable TA is any TA that induces or enhances an anti-tumor immune response in a host to whom the TA has been administered. Suitable TAs include, for example, species of gp100 (Cox et al., *Science*, 264:716-719 (1994); U.S. Pat. No. 6,500,919 B1 and WO 01/30847 with Val at residue 162, also referred to as "gp100M"; U.S. Pat. No. 6,537,560 B1 with Phe at residue 162), MART-1/Melan A (Kawakami et al., *J. Exp. Med.*, 180:347-352 (1994); U.S. Pat. No. 5,874,560), gp75 (TRP-1) (Wang et al., *J. Exp. Med.*, 186:1131-1140 (1996)), TRP-2 (Wang et al. 1996 J. Exp. Med. 184:2207; U.S. Pat. Nos. 5,831,016 and 6,083,783), tyrosinase (Wolfel et al., *Eur. J. Immunol.*, 24:759-764 (1994); WO 200175117; WO 200175016; WO 200175007), NY-ESO-1 (WO 98/14464; WO 99/18206; GenBank Accession No. P78358; U.S. Pat. No. 5,804,381), melanoma proteoglycan (Hellstrom et al., *J. Immunol.*, 130:1467-1472 (1983)), MAGE family antigens (i.e., MAGE-1, 2,3,4,6,12, 51; Van der Bruggen et al., *Science*, 254:1643-1647 (1991); U.S. Pat. Nos. 6,235,525; CN 1319611), BAGE family antigens (Boel et al., *Immunity*, 2:167-175 (1995)), GAGE family antigens (i.e., GAGE-1,2; Van den Eynde et al., *J. Exp. Med.*, 182:689-698 (1995); U.S. Pat. No. 6,013,765), RAGE family antigens (i.e., RAGE-1; Gaugler et at., *Immunogenetics*, 44:323-330 (1996); U.S. Pat. No. 5,939,526), N-acetylglucosaminyltransferase-V (Guilloux et at., *J. Exp. Med.*, 183:1173-1183 (1996)), p15 (Robbins et al., *J. Immunol.*

154:5944-5950 (1995)), ß-catenin (Robbins et al., *J. Exp. Med.*, 183:1185-1192 (1996)); MUM-1 (Coulie et al., *Proc. Natl. Acad. Sci.* USA, 92:7976-7980 (1995)), cyclin dependent kinase-4 (CDK4) (Wolfel et al., *Science*, 269:1281-1284 (1995)), p21-*ras* (Fossum et at., *Int. J. Cancer*, 56:40-45 (1994)), BCR-*abl* (Bocchia et al., *Blood*, 85:2680-2684 (1995)), p53 (Theobald et al., *Proc. Natl. Acad. Sci.* USA, 92:11993-11997 (1995)), p185 HER2/neu (erb-B1; Fisk et al., *J. Exp. Med.*, 181:2109-2117 (1995)), epidermal growth factor receptor (EGFR) (Harris et al., Breast Cancer Res. Treat, 29:1-2 (1994)), carcinoembryonic antigens (CEA) (Kwong et al., *J. Natl. Cancer Inst.*, 85:982-990 (1995) U.S. Pat. Nos. 5,756,103; 5,274,087; 5,571,710; 6,071,716; 5,698,530; 6,045,802; EP 263933; EP 346710; and, EP 784483); carcinoma-associated mutated mucins (i.e., MUC-1 gene products; Jerome et al., *J. Immunol.*, 151:1654-1662 (1993)); EBNA gene products of EBV (i.e., EBNA-1; Rickinson et al., *Cancer Surveys*, 13:53-80 (1992)); E7, E6 proteins of human papillomavirus (Ressing et al., *J. Immunol*, 154:5934-5943 (1995)); prostate specific antigen (PSA; Xue et al., *The Prostate*, 30:73-78 (1997)); prostate specific membrane antigen (PSMA; Israeli, et al., *Cancer Res.*, 54:1807-1811 (1994)); idiotypic epitopes or antigens, for example, immunoglobulin idiotypes or T cell receptor idiotypes (Chen et al., *J. Immunol.*, 153:4775-4787 (1994)); KSA (U.S. Patent No. 5,348,887), kinesin 2 (Dietz, et al. Biochem Biophys Res Commun 2000 Sep 7;275(3):731-8), HIP-55, TGFβ-1 anti-apoptotic factor (Toomey, et al. Br J Biomed Sci 2001;58(3):177-83), tumor protein D52 (Bryne J.A., et al., Genomics, 35:523-532 (1996)), H1FT, NY-BR-1 (WO 01/47959), NY-BR-62, NY-BR-75, NY-BR-85, NY-BR-87, NY-BR-96 (Scanlan, M. Serologic and Bioinformatic Approaches to the Identification of Human Tumor Antigens, in *Cancer Vaccines 2000*, Cancer Research Institute, New York, NY), including "wild-type" (i.e., normally encoded by the genome, naturally-occurring), modified, and mutated versions as well as other fragments and derivatives thereof. Any of these TAs may be utilized alone or in combination with one another in a co-immunization protocol.

Preferred TAs are useful for inducing an immune response against melanoma cells. The term "melanoma" includes but is not limited to melanomas, metastatic melanomas, melanomas derived from either melanocytes or melanocyte related nevus cells, melanocarcinomas, melanoepitheliomas, melanosarcomas, melanoma *in situ*, superficial spreading melanoma, nodular melanoma, lentigo maligna melanoma, acral lentiginous melanoma, invasive melanoma and familial atypical mole and melanoma (FAM-M) syndrome, for example. In general,

melanomas result from chromosomal abnormalities, degenerative growth and development disorders, mitogenic agents, ultraviolet radiation (UV), viral infections, inappropriate tissue expression of a gene, alterations in expression of a gene or carcinogenic agents, for example.

In certain cases, it may be beneficial to co-immunize patients with both TA and other antigens, such as angiogenesis-associated antigens ("AA"). An AA is an immunogenic molecule (i.e., peptide, polypeptide) associated with cells involved in the induction and / or continued development of blood vessels. For example, an AA may be expressed on an endothelial cell ("EC"), which is a primary structural component of blood vessels. Where the cancer is cancer, it is preferred that that the AA be found within or near blood vessels that supply a tumor. Immunization of a patient against an AA preferably results in an anti-AA immune response whereby angiogenic processes that occur near or within tumors are prevented and / or inhibited. Exemplary AAs include, for example, vascular endothelial growth factor (i.e., VEGF; Bernardini, et al. *J. Urol.*, 2001, 166(4): 1275-9; Starnes, et al. *J. Thorac. Cardiovasc. Surg.*, 2001, 122(3): 518-23; Dias, et al. *Blood*, 2002, 99: 2179-2184), the VEGF receptor (i.e., VEGF-R, flk-1/KDR; Starnes, et al. *J. Thorac. Cardiovasc. Surg.*, 2001, 122(3): 518-23), EPH receptors (i.e., EPHA2; Gerety, et al. 1999, *Cell*, 4: 403-414), epidermal growth factor receptor (i.e., EGFR; Ciardeillo, et al. Clin. Cancer Res., 2001, 7(10): 2958-70), basic fibroblast growth factor (i.e., bFGF; Davidson, et al. Clin. Exp. Metastasis 2000,18(6): 501-7; Poon, et al. Am J. Surg., 2001, 182(3):298-304), platelet-derived cell growth factor (i.e., PDGF-B), platelet-derived endothelial cell growth factor (PD-ECGF; Hong, et al. J. Mol. Med., 2001, 8(2):141-8), transforming growth factors (i.e., TGF-α; Hong, et al. J. Mol. Med., 2001, 8(2):141-8), endoglin (Balza, et al. *Int. J. Cancer*, 2001, 94: 579-585), Id proteins (Benezra, R. Trends Cardiovasc. Med., 2001, 11(6):237-41), proteases such as uPA, uPAR, and matrix metalloproteinases (MMP-2, MMP-9; Djonov, et al. J. Pathol., 2001, 195(2):147-55), nitric oxide synthase (Am. J. Ophthalmol., 2001, 132(4):551-6), aminopeptidase (Rouslhati, E. Nature Cancer, 2: 84-90, 2002), thrombospondins (i.e., TSP-1, TSP-2; Alvarez, et al. Gynecol. Oncol., 2001, 82(2):273-8; Seki, et al. Int. J. Oncol., 2001, 19(2):305-10), *k-ras* (Zhang, et al. Cancer Res., 2001, 61(16):6050-4), *Wnt* (Zhang, et al. Cancer Res., 2001, 61(16):6050-4), cyclin-dependent kinases (CDKs; Drug Resist. Updat. 2000, 3(2):83-88), microtubules (Timar, et al. 2001. *Path. Oncol. Res.*, 7(2): 85-94), heat shock proteins (i.e., HSP90 (Timar, *supra*)), heparin-binding factors (i.e., heparinase; Gohji, et al. Int. J. Cancer, 2001, 95(5):295-301), synthases (i.e., ATP synthase,

thymidilate synthase), collagen receptors, integrins (i.e., αυβ3, αυβ5, α1β1, α2β1, α5β1), the surface proteolglycan NG2, AAC2-1, or AAC2-2, among others, including "wild-type" (i.e., normally encoded by the genome, naturally-occurring), modified, mutated versions as well as other fragments and derivatives thereof. Any of these targets may be suitable in practicing the present invention, either alone or in combination with one another or with other agents.

The nucleic acid molecule may comprise or consist of a nucleotide sequence encoding one or more immunogenic targets, or fragments or derivatives thereof, such as that contained in a DNA insert in an ATCC Deposit. The term "nucleic acid sequence" or "nucleic acid molecule" refers to a DNA or RNA sequence. The term encompasses molecules formed from any of the known base analogs of DNA and RNA such as, but not limited to 4-acetylcytosine, 8-hydroxy-N6-methyladenosine, aziridinyl-cytosine, pseudoisocytosine, 5-(carboxyhydroxylmethyl) uracil, 5-fluorouracil, 5-bromouracil, 5-carboxymethylaminomethyl-2-thiouracil, 5-carboxy-methylaminomethyluracil, dihydrouracil, inosine, N6-iso-pentenyladenine, 1-methyladenine, 1-methylpseudouracil, 1-methylguanine, 1-methylinosine, 2,2-dimethyl-guanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyamino-methyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarbonyl-methyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, oxybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, N-uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid, pseudouracil, queosine, 2-thiocytosine, and 2,6-diaminopurine, among others.

An isolated nucleic acid molecule is one that: (1) is separated from at least about 50 percent of proteins, lipids, carbohydrates, or other materials with which it is naturally found when total nucleic acid is isolated from the source cells; (2) is not be linked to all or a portion of a polynucleotide to which the nucleic acid molecule is linked in nature; (3) is operably linked to a polynucleotide which it is not linked to in nature; and / or, (4) does not occur in nature as part of a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use. As used herein, the term "naturally occurring" or "native" or "naturally found" when used in connection

with biological materials such as nucleic acid molecules, polypeptides, host cells, and the like, refers to materials which are found in nature and are not manipulated by man. Similarly, "non-naturally occurring" or "non-native" as used herein refers to a material that is not found in nature or that has been structurally modified or synthesized by man.

The identity of two or more nucleic acid or amino acid sequences is determined by comparing the sequences. As known in the art, "identity" means the degree of sequence relatedness between nucleic acid or amino acid sequences as determined by the match between the units making up the molecules (i.e., nucleotides or amino acid residues). Identity measures the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., an algorithm). Identity between nucleic acid sequences may also be determined by the ability of the nucleic acid sequences to hybridize to one another. In defining the process of hybridization, the term "highly stringent conditions" and "moderately stringent conditions" refer to conditions that permit hybridization of nucleic acid strands whose sequences are complementary, and to exclude hybridization of significantly mismatched nucleic acids. Examples of "highly stringent conditions" for hybridization and washing are 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 50% formamide at 42°C. (see, for example, Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual* (2nd ed., Cold Spring Harbor Laboratory, 1989); Anderson *et al.*, *Nucleic Acid Hybridisation: A Practical Approach* Ch. 4 (IRL Press Limited)). The term "moderately stringent conditions" refers to conditions under which a DNA duplex with a greater degree of base pair mismatching than could occur under "highly stringent conditions" is able to form. Exemplary moderately stringent conditions are 0.015 M sodium chloride, 0.0015 M sodium citrate at 50-65°C or 0.015 M sodium chloride, 0.0015 M sodium citrate, and 20% formamide at 37-50°C. By way of example, moderately stringent conditions of 50°C in 0.015 M sodium ion will allow about a 21% mismatch. During hybridization, other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate, $NaDodSO_4$, (SDS), ficoll, Denhardt's solution, sonicated salmon sperm DNA (or another non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these

additives can be changed without substantially affecting the stringency of the hybridization conditions.. Hybridization experiments are usually carried out at pH 6.8-7.4; however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH.

In preferred embodiments of the present invention, vectors are used to transfer a nucleic acid sequence encoding an immunogenic target to a cell. A vector is any molecule used to transfer a nucleic acid sequence to a host cell. In certain cases, an expression vector is utilized. An expression vector is a nucleic acid molecule that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and / or control the expression of the transferred nucleic acid sequences. Expression includes, but is not limited to, processes such as transcription, translation, and splicing, if introns are present. Expression vectors typically comprise one or more flanking sequences operably linked to a heterologous nucleic acid sequence encoding a polypeptide. Flanking sequences may be homologous (i.e., from the same species and / or strain as the host cell), heterologous (i.e., from a species other than the host cell species or strain), hybrid (i.e., a combination of flanking sequences from more than one source), or synthetic, for example.

A flanking sequence is preferably capable of effecting the replication, transcription and / or translation of the coding sequence and is operably linked to a coding sequence. As used herein, the term operably linked refers to a linkage of polynucleotide elements in a functional relationship. For instance, a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the coding sequence. However, a flanking sequence need not necessarily be contiguous with the coding sequence, so long as it functions correctly. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the coding sequence and the promoter sequence may still be considered operably linked to the coding sequence. Similarly, an enhancer sequence may be located upstream or downstream from the coding sequence and affect transcription of the sequence.

In certain embodiments, it is preferred that the flanking sequence is a transcriptional regulatory region that drives high-level gene expression in the target cell. The transcriptional regulatory region may comprise, for example, a promoter, enhancer, silencer, repressor element, or combinations thereof. The transcriptional regulatory region may be either constitutive, tissue-specific, cell-type specific (i.e., the region is drives higher levels of transcription in a one type of tissue or cell as compared to another), or regulatable (i.e., responsive to interaction with a

compound such as tetracycline). The source of a transcriptional regulatory region may be any prokaryotic or eukaryotic organism, any vertebrate or invertebrate organism, or any plant, provided that the flanking sequence functions in a cell by causing transcription of a nucleic acid within that cell. A wide variety of transcriptional regulatory regions may be utilized in practicing the present invention.

Suitable transcriptional regulatory regions include the CMV promoter (i.e., the CMV-immediate early promoter); promoters from eukaryotic genes (i.e., the estrogen-inducible chicken ovalbumin gene, the interferon genes, the gluco-corticoid-inducible tyrosine aminotransferase gene, and the thymidine kinase gene); and the major early and late adenovirus gene promoters; the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-10); the promoter contained in the 3' long terminal repeat (LTR) of Rous sarcoma virus (RSV) (Yamamoto, *et al.*, 1980, *Cell* 22:787-97); the herpes simplex virus thymidine kinase (HSV-TK) promoter (Wagner *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:1444-45); the regulatory sequences of the metallothionine gene (Brinster *et al.*, 1982, *Nature* 296:39-42); prokaryotic expression vectors such as the beta-lactamase promoter (Villa-Kamaroff *et al.*, 1978, *Proc. Natl. Acad. Sci. U.S.A.*, 75:3727-31); or the tac promoter (DeBoer *et al.*, 1983, *Proc. Natl. Acad. Sci. U.S.A.*, 80:21-25). Tissue- and / or cell-type specific transcriptional control regions include, for example, the elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, *Cell* 38:639-46; Ornitz *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409 (1986); MacDonald, 1987, *Hepatology* 7:425-515); the insulin gene control region which is active in pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-22); the immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, *Cell* 38:647-58; Adames *et al.*, 1985, *Nature* 318:533-38; Alexander *et al.*, 1987, *Mol. Cell. Biol.*, 7:1436-44); the mouse mammary tumor virus control region in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, *Cell* 45:485-95); the albumin gene control region in liver (Pinkert *et al.*, 1987, *Genes and Devel.* 1:268-76); the alpha-feto-protein gene control region in liver (Krumlauf *et al.*, 1985, *Mol. Cell. Biol.*, 5:1639-48; Hammer *et al.*, 1987, *Science* 235:53-58); the alpha 1-antitrypsin gene control region in liver (Kelsey *et al.*, 1987, *Genes and Devel.* 1:161-71); the beta-globin gene control region in myeloid cells (Mogram *et al.*, 1985, *Nature* 315:338-40; Kollias *et al.*, 1986, *Cell* 46:89-94); the myelin basic protein gene control region in oligodendrocyte cells in the brain (Readhead *et al.*, 1987, *Cell* 48:703-12); the myosin light chain-2 gene control region in

skeletal muscle (Sani, 1985, *Nature* 314:283-86); the gonadotropic releasing hormone gene control region in the hypothalamus (Mason *et al.*, 1986, *Science* 234:1372-78), and the tyrosinase promoter in melanoma cells (Hart, I. Semin Oncol 1996 Feb;23(1):154-8; Siders, et al. Cancer Gene Ther 1998 Sep-Oct;5(5):281-91), among others. Inducible promoters that are activated in the presence of a certain compound or condition such as light, heat, radiation, tetracycline, or heat shock proteins, for example, may also be utilized (see, for example, WO 00/10612). Other suitable promoters are known in the art.

As described above, enhancers may also be suitable flanking sequences. Enhancers are cis-acting elements of DNA, usually about 10-300 bp in length, that act on the promoter to increase transcription. Enhancers are typically orientation- and position-independent, having been identified both 5' and 3' to controlled coding sequences. Several enhancer sequences available from mammalian genes are known (i.e., globin, elastase, albumin, alpha-feto-protein and insulin). Similarly, the SV40 enhancer, the cytomegalovirus early promoter enhancer, the polyoma enhancer, and adenovirus enhancers are useful with eukaryotic promoter sequences. While an enhancer may be spliced into the vector at a position 5' or 3' to nucleic acid coding sequence, it is typically located at a site 5' from the promoter. Other suitable enhancers are known in the art, and would be applicable to the present invention.

While preparing reagents of the present invention, cells may need to be transfected or transformed. Transfection refers to the uptake of foreign or exogenous DNA by a cell, and a cell has been transfected when the exogenous DNA has been introduced inside the cell membrane. A number of transfection techniques are well known in the art (i.e., Graham *et al.*, 1973, *Virology* 52:456; Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratories, 1989); Davis *et al.*, *Basic Methods in Molecular Biology* (Elsevier, 1986); and Chu *et al.*, 1981, *Gene* 13:197). Such techniques can be used to introduce one or more exogenous DNA moieties into suitable host cells.

In certain embodiments, it is preferred that transfection of a cell results in transformation of that cell. A cell is transformed when there is a change in a characteristic of the cell, being transformed when it has been modified to contain a new nucleic acid. Following transfection, the transfected nucleic acid may recombine with that of the cell by physically integrating into a chromosome of the cell, may be maintained transiently as an episomal element without being

replicated, or may replicate independently as a plasmid. A cell is stably transformed when the nucleic acid is replicated with the division of the cell.

The expression vectors of the present invention also provide for expression of fragments of immunogenic targets. Fragments may include sequences truncated at the amino terminus (with or without a leader sequence) and / or the carboxy terminus. Fragments may also include variants (i.e., allelic, splice), orthologs, homologues, and other variants having one or more amino acid additions or substitutions or internal deletions as compared to the parental sequence. In preferred embodiments, truncations and/or deletions comprise about 1-5 amino acids, 5-10 amino acids, 10-20 amino acids, 20-30 amino acids, 30-40 amino acids, 40-50 amino acids, or more. Such polypeptide fragments may optionally comprise an amino terminal methionine residue. It will be appreciated that such fragments can be used, for example, to generate antibodies or cellular immune responses to immunogenic targets.

A variant is a sequence having one or more sequence substitutions, deletions, and/or additions as compared to the subject sequence. Variants may be naturally occurring or artificially constructed. Such variants may be prepared from the corresponding nucleic acid molecules. In preferred embodiments, the variants have from 1 to 3, or from 1 to 5, or from 1 to 10, or from 1 to 15, or from 1 to 20, or from 1 to 25, or from 1 to 30, or from 1 to 40, or from 1 to 50, or more than 50 amino acid substitutions, insertions, additions and/or deletions.

An allelic variant is one of several possible naturally-occurring alternate forms of a sequence occupying a given locus on a chromosome of an organism or a population of organisms. A splice variant is a polypeptide generated from one of several RNA transcript resulting from splicing of a primary transcript. An ortholog is a similar nucleic acid or polypeptide sequence from another species. For example, the mouse and human versions of an immunogenic target may be considered orthologs of each other. A derivative of a sequence is one that is derived from a parental sequence those sequences having substitutions, additions, deletions, or chemically modified variants. Variants may also include fusion proteins, which refers to the fusion of one or more first sequences (such as a peptide) at the amino or carboxy terminus of at least one other sequence (such as a heterologous peptide).

"Similarity" is a concept related to identity, except that similarity refers to a measure of relatedness which includes both identical matches and conservative substitution matches. If two polypeptide sequences have, for example, 10/20 identical amino acids, and the remainder are all

non-conservative substitutions, then the percent identity and similarity would both be 50%. If in the same example, there are five more positions where there are conservative substitutions, then the percent identity remains 50%, but the percent similarity would be 75% (15/20). Therefore, in cases where there are conservative substitutions, the percent similarity between two polypeptides will be higher than the percent identity between those two polypeptides.

Substitutions may be conservative, or non-conservative, or any combination thereof. Conservative amino acid modifications to the sequence of a polypeptide (and the corresponding modifications to the encoding nucleotides) may produce polypeptides having functional and chemical characteristics similar to those of a parental polypeptide. For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a non-native residue such that there is little or no effect on the size, polarity, charge, hydrophobicity, or hydrophilicity of the amino acid residue at that position and, in particular, does not result in decreased immunogenicity. Suitable conservative amino acid substitutions are shown in **Table I**.

Table I

Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala	Val, Leu, Ile	Val
Arg	Lys, Gln, Asn	Lys
Asn	Gln	Gln
Asp	Glu	Glu
Cys	Ser, Ala	Ser
Gln	Asn	Asn
Glu	Asp	Asp
Gly	Pro, Ala	Ala
His	Asn, Gln, Lys, Arg	Arg
Ile	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys	Arg, 1,4 Diamino-butyric Acid, Gln, Asn	Arg
Met	Leu, Phe, Ile	Leu
Phe	Leu, Val, Ile, Ala, Tyr	Leu
Pro	Ala	Gly
Ser	Thr, Ala, Cys	Thr
Thr	Ser	Ser
Trp	Tyr, Phe	Tyr
Tyr	Trp, Phe, Thr, Ser	Phe
Val	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

A skilled artisan will be able to determine suitable variants of an immunogenic target using well-known techniques. For identifying suitable areas of the molecule that may be changed without destroying biological activity (i.e., MHC binding, immunogenicity), one skilled in the art may target areas not believed to be important for that activity. For example, when immunogenic targets with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a polypeptide to such similar polypeptides. By performing such analyses, one can identify residues and portions of the molecules that are conserved. It will be appreciated that changes in areas of the molecule that are not conserved relative to such similar immunogenic targets would be less likely to adversely affect the biological activity and/or structure of a polypeptide. Similarly, the residues required for binding to MHC are known, and may be modified to improve binding. However, modifications resulting in decreased binding to MHC will not be appropriate in most situations. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically similar amino acids for the naturally occurring residues while retaining activity. Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the structure of the immunogenic target.

Other preferred polypeptide variants include glycosylation variants wherein the number and/or type of glycosylation sites have been altered compared to the subject amino acid sequence. In one embodiment, polypeptide variants comprise a greater or a lesser number of N-linked glycosylation sites than the subject amino acid sequence. An N-linked glycosylation site is characterized by the sequence Asn-X-Ser or Asn-X-Thr, wherein the amino acid residue designated as X may be any amino acid residue except proline. The substitution of amino acid residues to create this sequence provides a potential new site for the addition of an N-linked carbohydrate chain. Alternatively, substitutions that eliminate this sequence will remove an existing N-linked carbohydrate chain. Also provided is a rearrangement of N-linked carbohydrate chains wherein one or more N-linked glycosylation sites (typically those that are naturally occurring) are eliminated and one or more new N-linked sites are created. To affect O-linked glycosylation of a polypeptide, one would modify serine and / or threonine residues.

Additional preferred variants include cysteine variants, wherein one or more cysteine residues are deleted or substituted with another amino acid (*e.g.*, serine) as compared to the subject amino acid sequence set. Cysteine variants are useful when peptides or polypeptides must be refolded into a biologically active conformation such as after the isolation of insoluble inclusion bodies. Cysteine variants generally have fewer cysteine residues than the native protein, and typically have an even number to minimize interactions resulting from unpaired cysteines.

In other embodiments, the peptides or polypeptides may be attached to one or more fusion segments that assist in purification of the polypeptides. Fusions can be made either at the amino terminus or at the carboxy terminus of the subject polypeptide variant thereof. Fusions may be direct with no linker or adapter molecule or may be through a linker or adapter molecule. A linker or adapter molecule may be one or more amino acid residues, typically from about 20 to about 50 amino acid residues. A linker or adapter molecule may also be designed with a cleavage site for a DNA restriction endonuclease or for a protease to allow for the separation of the fused moieties. It will be appreciated that once constructed, the fusion polypeptides can be derivatized according to the methods described herein. Suitable fusion segments include, among others, metal binding domains (e.g., a poly-histidine segment), immunoglobulin binding domains (i.e., Protein A, Protein G, T cell, B cell, Fc receptor, or complement protein antibody-binding domains), sugar binding domains (e.g., a maltose binding domain), and/or a "tag" domain (i.e., at least a portion of α-galactosidase, a strep tag peptide, a T7 tag peptide, a FLAG peptide, or other domains that can be purified using compounds that bind to the domain, such as monoclonal antibodies). This tag is typically fused to the peptide or polypeptide and upon expression may serve as a means for affinity purification of the sequence of interest polypeptide from the host cell. Affinity purification can be accomplished, for example, by column chromatography using antibodies against the tag as an affinity matrix. Optionally, the tag can subsequently be removed from the purified sequence of interest polypeptide by various means such as using certain peptidases for cleavage. As described below, fusions may also be made between a TA and a co-stimulatory components such as the chemokines CXC10 (IP-10), CCL7 (MCP-3), or CCL5 (RANTES), for example.

A fusion motif may enhance transport of an immunogenic target to an MHC processing compartment, such as the endoplasmic reticulum. These sequences, referred to as tranduction or

transcytosis sequences, include sequences derived from HIV tat (see Kim et al. 1997 J. Immunol. 159:1666), *Drosophila* antennapedia (see Schutze-Redelmeier et al. 1996 J. Immunol. 157:650), or human period-1 protein (hPER1; in particular, SRRHHCRSKAKRSRHH).

In addition, the polypeptide or variant thereof may be fused to a homologous peptide or polypeptide to form a homodimer or to a heterologous peptide or polypeptide to form a heterodimer. Heterologous peptides and polypeptides include, but are not limited to an epitope to allow for the detection and/or isolation of a fusion polypeptide; a transmembrane receptor protein or a portion thereof, such as an extracellular domain or a transmembrane and intracellular domain; a ligand or a portion thereof which binds to a transmembrane receptor protein; an enzyme or portion thereof which is catalytically active; a polypeptide or peptide which promotes oligomerization, such as a leucine zipper domain; a polypeptide or peptide which increases stability, such as an immunoglobulin constant region; a peptide or polypeptide which has a therapeutic activity different from the peptide or polypeptide; and/or variants thereof.

In certain embodiments, it may be advantageous to combine a nucleic acid sequence encoding an immunogenic target with one or more co-stimulatory component(s) such as cell surface proteins, cytokines or chemokines in a composition of the present invention. The co-stimulatory component may be included in the composition as a polypeptide or as a nucleic acid encoding the polypeptide, for example. Suitable co-stimulatory molecules include, for instance, polypeptides that bind members of the CD28 family (i.e., CD28, ICOS; Hutloff, et al. *Nature* 1999, 397: 263–265; Peach, et al. *J Exp Med* 1994, 180: 2049–2058) such as the CD28 binding polypeptides B7.1 (CD80; Schwartz, 1992; Chen et al, 1992; Ellis, et al. *J. Immunol.*, 156(8): 2700-9), B7.2 (CD86; Ellis, et al. *J. Immunol.*, 156(8): 2700-9), and mutants / variants thereof (WO 00/66162); polypeptides which bind members of the integrin family (i.e., LFA-1 (CD11a / CD18); Sedwick, et al. *J Immunol* 1999, 162: 1367–1375; Wülfing, et al. *Science* 1998, 282: 2266–2269; Lub, et al. *Immunol Today* 1995, 16: 479–483) including members of the ICAM family (i.e., ICAM-1, -2 or -3); polypeptides which bind CD2 family members (i.e., CD2, signalling lymphocyte activation molecule (CDw150 or "SLAM"; Aversa, et al. *J Immunol* 1997, 158: 4036–4044)) such as CD58 (LFA-3; CD2 ligand; Davis, et al. *Immunol Today* 1996, 17: 177–187) or SLAM ligands (Sayos, et al. *Nature* 1998, 395: 462–469); polypeptides which bind heat stable antigen (HSA or CD24; Zhou, et al. *Eur J Immunol* 1997, 27: 2524–2528); polypeptides which bind to members of the TNF receptor (TNFR) family (i.e.,

4-1BB (CD137; Vinay, et al. *Semin Immunol* 1998, 10: 481–489), OX40 (CD134; Weinberg, et al. *Semin Immunol* 1998, 10: 471–480; Higgins, et al. *J Immunol* 1999, 162: 486–493), and CD27 (Lens, et al. *Semin Immunol* 1998, 10: 491–499)) such as 4-1BBL (4-1BB ligand; Vinay, et al. *Semin Immunol* 1998, 10: 481–48; DeBenedette, et al. *J Immunol* 1997, 158: 551–559), TNFR associated factor-1 (TRAF-1; 4-1BB ligand; Saoulli, et al. *J Exp Med* 1998, 187: 1849–1862, Arch, et al. *Mol Cell Biol* 1998, 18: 558–565), TRAF-2 (4-1BB and OX40 ligand; Saoulli, et al. *J Exp Med* 1998, 187: 1849–1862; Oshima, et al. *Int Immunol* 1998, 10: 517–526, Kawamata, et al. *J Biol Chem* 1998, 273: 5808–5814), TRAF-3 (4-1BB and OX40 ligand; Arch, et al. *Mol Cell Biol* 1998, 18: 558–565; Jang, et al. *Biochem Biophys Res Commun* 1998, 242: 613–620; Kawamata S, et al. *J Biol Chem* 1998, 273: 5808–5814), OX40L (OX40 ligand; Gramaglia, et al. *J Immunol* 1998, 161: 6510–6517), TRAF-5 (OX40 ligand; Arch, et al. *Mol Cell Biol* 1998, 18: 558–565; Kawamata, et al. *J Biol Chem* 1998, 273: 5808–5814), and CD70 (CD27 ligand; Couderc, et al. *Cancer Gene Ther.*, 5(3): 163-75). CD154 (CD40 ligand or "CD40L"; Gurunathan, et al. *J. Immunol.*, 1998, 161: 4563-4571; Sine, et al. *Hum. Gene Ther.*, 2001, 12: 1091-1102) may also be suitable.

One or more cytokines may also be suitable co-stimulatory components or "adjuvants", either as polypeptides or being encoded by nucleic acids contained within the compositions of the present invention (Parmiani, et al. Immunol Lett 2000 Sep 15; 74(1): 41-4; Berzofsky, et al. Nature Immunol. 1: 209-219). Suitable cytokines include, for example, interleukin-2 (IL-2) (Rosenberg, et al. *Nature Med.* 4: 321-327 (1998)), IL-4, IL-7, IL-12 (reviewed by Pardoll, 1992; Harries, et al. J. Gene Med. 2000 Jul-Aug;2(4):243-9; Rao, et al. *J. Immunol.* 156: 3357-3365 (1996)), IL-15 (Xin, et al. *Vaccine*, 17:858-866, 1999), IL-16 (Cruikshank, et al. J. Leuk Biol. 67(6): 757-66, 2000), IL-18 (*J. Cancer Res. Clin. Oncol.* 2001. 127(12): 718-726), GM-CSF (CSF (Disis, et al. *Blood*, 88: 202-210 (1996)), tumor necrosis factor-alpha (TNF-α), or interferons such as IFN-α or INF-γ. Other cytokines may also be suitable for practicing the present invention, as is known in the art.

Chemokines may also be utilized, in either polypeptide or nucleic acid form. Fusion proteins comprising CXCL10 (IP-10) and CCL7 (MCP-3) fused to a tumor self-antigen have been shown to induce anti-tumor immunity (Biragyn, et al. *Nature Biotech.* 1999, 17: 253-258). The chemokines CCL3 (MIP-1α) and CCL5 (RANTES) (Boyer, et al. *Vaccine*, 1999, 17 (Supp.

2): S53-S64) may also be of use in practicing the present invention. Other suitable chemokines are known in the art.

It is also known in the art that suppressive or negative regulatory immune mechanisms may be blocked, resulting in enhanced immune responses. For instance, treatment with anti-CTLA-4 (Shrikant, et al. *Immunity*, 1996, 14: 145-155; Sutmuller, et al. *J. Exp. Med.*, 2001, 194: 823-832), anti-CD25 (Sutmuller, *supra*), anti-CD4 (Matsui, et al. *J. Immunol.*, 1999, 163: 184-193), the fusion protein IL13Ra2-Fc (Terabe, et al. *Nature Immunol.*, 2000, 1: 515-520), and combinations thereof (i.e., anti-CTLA-4 and anti-CD25, Sutmuller, *supra*) have been shown to upregulate anti-tumor immune responses and would be suitable in practicing the present invention. Such treatments, among others, may also be combined with the one or more immunogenic targets of the present invention.

Any of these components may be used alone or in combination with other agents. For instance, it has been shown that a combination of CD80, ICAM-1 and LFA-3 ("TRICOM") may potentiate anti-cancer immune responses (Hodge, et al. *Cancer Res.* 59: 5800-5807 (1999). Other effective combinations include, for example, IL-12 + GM-CSF (Ahlers, et al. *J. Immunol.*, 158: 3947-3958 (1997); Iwasaki, et al. *J. Immunol.* 158: 4591-4601 (1997)), IL-12 + GM-CSF + TNF-α (Ahlers, et al. *Int. Immunol.* 13: 897-908 (2001)), CD80 + IL-12 (Fruend, et al. *Int. J. Cancer*, 85: 508-517 (2000); Rao, et al. *supra*), and CD86 + GM-CSF + IL-12 (Iwasaki, supra). One of skill in the art would be aware of additional combinations useful in carrying out the present invention. In addition, the skilled artisan would be aware of additional reagents or methods that may be used to modulate such mechanisms. These reagents and methods, as well as others known by those of skill in the art, may be utilized in practicing the present invention.

Additional strategies for improving the efficiency of nucleic acid-based immunization may also be used including, for example, the use of self-replicating viral replicons (Caley, et al. 1999. *Vaccine*, 17: 3124-2135; Dubensky, et al. 2000. *Mol. Med.* 6: 723-732; Leitner, et al. 2000. *Cancer Res.* 60: 51-55), codon optimization (Liu, et al. 2000. *Mol. Ther.*, 1: 497-500; Dubensky, *supra*; Huang, et al. 2001. *J. Virol.* 75: 4947-4951), *in vivo* electroporation (Widera, et al. 2000. *J. Immunol.* 164: 4635-3640), incorporation of CpG stimulatory motifs (Gurunathan, et al. *Ann. Rev. Immunol.*, 2000, 18: 927-974; Leitner, *supra*; Cho, et al. J. Immunol. 168(10):4907-13), sequences for targeting of the endocytic or ubiquitin-processing pathways (Thomson, et al. 1998. *J. Virol.* 72: 2246-2252; Velders, et al. 2001. *J. Immunol.*

166: 5366-5373), Marek's disease virus type 1 VP22 sequences (J. Virol. 76(6):2676-82, 2002), prime-boost regimens (Gurunathan, *supra*; Sullivan, et al. 2000. *Nature*, 408: 605-609; Hanke, et al. 1998. *Vaccine*, 16: 439-445; Amara, et al. 2001. *Science*, 292: 69-74), and the use of mucosal delivery vectors such as *Salmonella* (Darji, et al. 1997. *Cell*, 91: 765-775; Woo, et al. 2001. *Vaccine*, 19: 2945-2954). Other methods are known in the art, some of which are described below.

Chemotherapeutic agents, radiation, anti-angiogenic compounds, or other agents may also be utilized in treating and / or preventing cancer using immunogenic targets (Sebti, et al. Oncogene 2000 Dec 27;19(56):6566-73). For example, in treating metastatic melanoma, suitable chemotherapeutic regimens may include BELD (bleomycin, vindesine, lomustine, and deacarbazine; Young, et al. 1985. Cancer, 55: 1879-81), BOLD (bleomycin, vincristine, lomustine, dacarbazine; Seigler, et al. 1980. Cancer, 46 : 2346-8) ; DD (dacarbazine, actinomycin ; Hochster, et al. Cancer Treatment Reports, 69: 39-42), or POC (procarbazine, vincristine, lomustine; Carmo-Pereira, et al. 1984. Cancer Treatment Reports, 68: 1211-4) among others. Other suitable chemotherapeutic regimens may also be utilized.

Many anti-angiogenic agents are known in the art and would be suitable for co-administration with the immunogenic target vaccines and/or chemotherapeutic regimens (see, for example, Timar, et al. 2001. *Pathology Oncol. Res.*, 7(2): 85-94). Such agents include, for example, physiological agents such as growth factors (i.e., ANG-2, NK1,2,4 (HGF), transforming growth factor beta (TGF-β)), cytokines (i.e., interferons such as IFN-α, -β, -γ, platelet factor 4 (PF-4), PR-39), proteases (i.e., cleaved AT-III, collagen XVIII fragment (Endostatin)), HmwKallikrein-d5 plasmin fragment (Angiostatin), prothrombin-F1-2, TSP-1), protease inhibitors (i.e., tissue inhibitor of metalloproteases such as TIMP-1, -2, or –3; maspin; plasminogen activator-inhibitors such as PAI-1; pigment epithelium derived factor (PEDF)), Tumstatin (available through ILEX, Inc.), antibody products (i.e., the collagen-binding antibodies HUIV26, HUI77, XL313; anti-VEGF; anti-integrin (i.e., Vitaxin, (Lxsys))), and glycosidases (i.e., heparinase-I, -III). "Chemical" or modified physiological agents known or believed to have anti-angiogenic potential include, for example, vinblastine, taxol, ketoconazole, thalidomide, dolestatin, combrestatin A, rapamycin (Guba, et al. 2002, *Nature Med.*, 8: 128-135), CEP-7055 (available from Cephalon, Inc.), flavone acetic acid, Bay 12-9566 (Bayer Corp.), AG3340 (Agouron, Inc.), CGS 27023A (Novartis), tetracylcine derivatives (i.e., COL-3

(Collagenix, Inc.)), Neovastat (Aeterna), BMS-275291 (Bristol-Myers Squibb), low dose 5-FU, low dose methotrexate (MTX), irsofladine, radicicol, cyclosporine, captopril, celecoxib, D45152-sulphated polysaccharide, cationic protein (Protamine), cationic peptide-VEGF, Suramin (polysulphonated napthyl urea), compounds that interfere with the function or production of VEGF (i.e., SU5416 or SU6668 (Sugen), PTK787/ZK22584 (Novartis)), Distamycin A, Angiozyme (ribozyme), isoflavinoids, staurosporine derivatives, genistein, EMD121974 (Merck KcgaA), tyrphostins, isoquinolones, retinoic acid, carboxyamidotriazole, TNP-470, octreotide, 2-methoxyestradiol, aminosterols (i.e., squalamine), glutathione analogues (i.e., N-acteyl-L-cysteine), combretastatin A-4 (Oxigene), Eph receptor blocking agents (*Nature*, 414:933-938, 2001), Rh-Angiostatin, Rh-Endostatin (WO 01/93897), cyclic-RGD peptide, accutin-disintegrin, benzodiazepenes, humanized anti-avb3 Ab, Rh-PAI-2, amiloride, p-amidobenzamidine, anti-uPA ab, anti-uPAR Ab, L-phanylalanin-N-methylamides (i.e., Batimistat, Marimastat), AG3340, and minocycline. Many other suitable agents are known in the art and would suffice in practicing the present invention.

The present invention may also be utilized in combination with "non-traditional" methods of treating cancer. For example, it has recently been demonstrated that administration of certain anaerobic bacteria may assist in slowing tumor growth. In one study, *Clostridium novyi* was modified to eliminate a toxin gene carried on a phage episome and administered to mice with colorectal tumors (Dang, et al. *P.N.A.S. USA*, 98(26): 15155-15160, 2001). In combination with chemotherapy, the treatment was shown to cause tumor necrosis in the animals. The reagents and methodologies described in this application may be combined with such treatment methodologies.

Nucleic acids encoding immunogenic targets may be administered to patients by any of several available techniques. Various viral vectors that have been successfully utilized for introducing a nucleic acid to a host include retrovirus, adenovirus, adeno-associated virus (AAV), herpes virus, and poxvirus, among others. It is understood in the art that many such viral vectors are available in the art. The vectors of the present invention may be constructed using standard recombinant techniques widely available to one skilled in the art. Such techniques may be found in common molecular biology references such as *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press,

San Diego, CA), and *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press, San Diego, CA).

Preferred retroviral vectors are derivatives of lentivirus as well as derivatives of murine or avian retroviruses. Examples of suitable retroviral vectors include, for example, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), SIV, BIV, HIV and Rous Sarcoma Virus (RSV). A number of retroviral vectors can incorporate multiple exogenous nucleic acid sequences. As recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided by, for example, helper cell lines encoding retrovirus structural genes. Suitable helper cell lines include Ψ2, PA317 and PA12, among others. The vector virions produced using such cell lines may then be used to infect a tissue cell line, such as NIH 3T3 cells, to produce large quantities of chimeric retroviral virions. Retroviral vectors may be administered by traditional methods (i.e., injection) or by implantation of a "producer cell line" in proximity to the target cell population (Culver, K., *et al.*, 1994, *Hum. Gene Ther.*, 5 (3): 343-79; Culver, K., *et al.*, *Cold Spring Harb. Symp. Quant. Biol.*, 59: 685-90); Oldfield, E., 1993, *Hum. Gene Ther.*, 4 (1): 39-69). The producer cell line is engineered to produce a viral vector and releases viral particles in the vicinity of the target cell. A portion of the released viral particles contact the target cells and infect those cells, thus delivering a nucleic acid of the present invention to the target cell. Following infection of the target cell, expression of the nucleic acid of the vector occurs.

Adenoviral vectors have proven especially useful for gene transfer into eukaryotic cells (Rosenfeld, M., *et al.*, 1991, *Science*, 252 (5004): 431-4; Crystal, R., *et al.*, 1994, *Nat. Genet.*, 8 (1): 42-51), the study eukaryotic gene expression (Levrero, M., *et al.*, 1991, *Gene*, 101 (2): 195-202), vaccine development (Graham, F. and Prevec, L., 1992, *Biotechnology*, 20: 363-90), and in animal models (Stratford-Perricaudet, L., *et al.*, 1992, *Bone Marrow Transplant.*, 9 (Suppl. 1): 151-2 ; Rich, D., *et al.*, 1993, *Hum. Gene Ther.*, 4 (4): 461-76). Experimental routes for administrating recombinant Ad to different tissues *in vivo* have included intratracheal instillation (Rosenfeld, M., *et al.*, 1992, *Cell*, 68 (1): 143-55) injection into muscle (Quantin, B., *et al.*, 1992, *Proc. Natl. Acad. Sci. U.S.A.*, 89 (7): 2581-4), peripheral intravenous injection (Herz, J., and Gerard, R., 1993, *Proc. Natl. Acad. Sci. U.S.A.*, 90 (7): 2812-6) and stereotactic inoculation to brain (Le Gal La Salle, G., *et al.*, 1993, *Science*, 259 (5097): 988-90), among others.

Adeno-associated virus (AAV) demonstrates high-level infectivity, broad host range and specificity in integrating into the host cell genome (Hermonat, P., et al., 1984, *Proc. Natl. Acad. Sci. U.S.A.*, 81 (20): 6466-70). And Herpes Simplex Virus type-1 (HSV-1) is yet another attractive vector system, especially for use in the nervous system because of its neurotropic property (Geller, A., *et al.*, 1991, *Trends Neurosci.*, 14 (10): 428-32; Glorioso, *et al.*, 1995, *Mol. Biotechnol.*, 4 (1): 87-99; Glorioso, *et al.*, 1995, *Annu. Rev. Microbiol.*, 49: 675-710).

Poxvirus is another useful expression vector (Smith, et al. 1983, *Gene*, 25 (1): 21-8; Moss, et al, 1992, *Biotechnology*, 20: 345-62; Moss, et al, 1992, *Curr. Top. Microbiol. Immunol.*, 158: 25-38; Moss, et al. 1991. *Science*, 252: 1662-1667). Poxviruses shown to be useful include vaccinia, NYVAC, avipox, fowlpox, canarypox, ALVAC, and ALVAC(2), among others.

NYVAC (vP866) was derived from the Copenhagen vaccine strain of vaccinia virus by deleting six nonessential regions of the genome encoding known or potential virulence factors (see, for example, U.S. Pat. Nos. 5,364,773 and 5,494,807). The deletion loci were also engineered as recipient loci for the insertion of foreign genes. The deleted regions are: thymidine kinase gene (TK; J2R); hemorrhagic region (u; B13R+B14R); A type inclusion body region (ATI; A26L); hemagglutinin gene (HA; A56R); host range gene region (C7L-K1L); and, large subunit, ribonucleotide reductase (I4L). NYVAC is a genetically engineered vaccinia virus strain that was generated by the specific deletion of eighteen open reading frames encoding gene products associated with virulence and host range. NYVAC has been show to be useful for expressing TAs (see, for example, U.S. Pat. No. 6,265,189). NYVAC (vP866), vP994, vCP205, vCP1433, placZH6H4Lreverse, pMPC6H6K3E3 and pC3H6FHVB were also deposited with the ATCC under the terms of the Budapest Treaty, accession numbers VR-2559, VR-2558, VR-2557, VR-2556, ATCC-97913, ATCC-97912, and ATCC-97914, respectively.

ALVAC-based recombinant viruses (i.e., ALVAC-1 and ALVAC-2) are also suitable for use in practicing the present invention (see, for example, U.S. Pat. No. 5,756,103). ALVAC(2) is identical to ALVAC(1) except that ALVAC(2) genome comprises the vaccinia E3L and K3L genes under the control of vaccinia promoters (U.S. Pat. No. 6,130,066; Beattie et al., 1995a, 1995b, 1991; Chang et al., 1992; Davies et al., 1993). Both ALVAC(1) and ALVAC(2) have been demonstrated to be useful in expressing foreign DNA sequences, such as TAs (Tartaglia et al., 1993 a,b; U.S. Pat. No. 5,833,975). ALVAC was deposited under the terms of the Budapest

Treaty with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110-2209, USA, ATCC accession number VR-2547.

Another useful poxvirus vector is TROVAC. TROVAC refers to an attenuated fowlpox that was a plaque-cloned isolate derived from the FP-1 vaccine strain of fowlpoxvirus which is licensed for vaccination of 1 day old chicks. TROVAC was likewise deposited under the terms of the Budapest Treaty with the ATCC, accession number 2553.

"Non-viral" plasmid vectors may also be suitable in practicing the present invention. Preferred plasmid vectors are compatible with bacterial, insect, and / or mammalian host cells. Such vectors include, for example, PCR-II, pCR3, and pcDNA3.1 (Invitrogen, San Diego, CA), pBSII (Stratagene, La Jolla, CA), pET15 (Novagen, Madison, WI), pGEX (Pharmacia Biotech, Piscataway, NJ), pEGFP-N2 (Clontech, Palo Alto, CA), pETL (BlueBacII, Invitrogen), pDSR-alpha (PCT pub. No. WO 90/14363) and pFastBacDual (Gibco-BRL, Grand Island, NY) as well as Bluescript® plasmid derivatives (a high copy number COLE1-based phagemid, Stratagene Cloning Systems, La Jolla, CA), PCR cloning plasmids designed for cloning Taq-amplified PCR products (*e.g.*, TOPO™ TA cloning® kit, PCR2.1® plasmid derivatives, Invitrogen, Carlsbad, CA). Bacterial vectors may also be used with the current invention. These vectors include, for example, *Shigella, Salmonella, Vibrio cholerae, Lactobacillus, Bacille calmette guérin (BCG), and Streptococcus* (see for example, WO 88/6626; WO 90/0594; WO 91/13157; WO 92/1796; and WO 92/21376). Many other non-viral plasmid expression vectors and systems are known in the art and could be used with the current invention.

Suitable nucleic acid delivery techniques include DNA-ligand complexes, adenovirus-ligand-DNA complexes, direct injection of DNA, $CaPO_4$ precipitation, gene gun techniques, electroporation, and colloidal dispersion systems, among others. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome, which are artificial membrane vesicles useful as delivery vehicles *in vitro* and *in vivo*. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, R., *et al.*, 1981, *Trends Biochem. Sci.*, 6: 77). The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in

combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

An immunogenic target may also be administered in combination with one or more adjuvants to boost the immune response. Exemplary adjuvants are shown in Table II below:

Table II

Types of Immunologic Adjuvants

Type of Adjuvant	General Examples	Specific Examples/References
Gel-type	Aluminum hydroxide/phosphate ("alum adjuvants")	(Aggerbeck and Heron, 1995)
	Calcium phosphate	(Relyveld, 1986)
Microbial	Muramyl dipeptide (MDP)	(Chedid et al., 1986)
	Bacterial exotoxins	Cholera toxin (CT), *E.coli* labile toxin (LT)(Freytag and Clements, 1999)
	Endotoxin-based adjuvants	Monophosphoryl lipid A (MPL) (Ulrich and Myers, 1995)
	Other bacterial	CpG oligonucleotides (Corral and Petray, 2000), BCG sequences (Krieg, et al. *Nature*, 374:576), tetanus toxoid (Rice, et al. *J. Immunol.*, 2001, 167: 1558-1565)
Particulate	Biodegradable Polymer microspheres	(Gupta et al., 1998)
	Immunostimulatory complexes (ISCOMs)	(Morein and Bengtsson, 1999)
	Liposomes	(Wassef et al., 1994)
Oil-emulsion and surfactant-based adjuvants	Freund's incomplete adjuvant	(Jensen et al., 1998)
	Microfluidized emulsions	MF59 (Ott et al., 1995)
		SAF (Allison and Byars, 1992) (Allison, 1999)
	Saponins	QS-21 (Kensil, 1996)
Synthetic	Muramyl peptide derivatives	Murabutide (Lederer, 1986) Threony-MDP (Allison, 1997)
	Nonionic block copolymers	L121 (Allison, 1999)
	Polyphosphazene (PCPP)	(Payne et al., 1995)

	Synthetic polynucleotides	Poly A:U, Poly I:C (Johnson, 1994)
	Thalidomide derivatives	CC-4047/ACTIMID (J. Immunol., 168(10):4914-9)

Administration of a composition of the present invention to a host may be accomplished using any of a variety of techniques known to those of skill in the art. The composition(s) may be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals (i.e., a "pharmaceutical composition"). The pharmaceutical composition is preferably made in the form of a dosage unit containing a given amount of DNA, viral vector particles, polypeptide or peptide, for example. A suitable daily dose for a human or other mammal may vary widely depending on the condition of the patient and other factors, but, once again, can be determined using routine methods.

The pharmaceutical composition may be administered orally, parentally, by inhalation spray, rectally, intranodally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term "pharmaceutically acceptable carrier" or "physiologically acceptable carrier" as used herein refers to one or more formulation materials suitable for accomplishing or enhancing the delivery of a nucleic acid, polypeptide, or peptide as a pharmaceutical composition. A "pharmaceutical composition" is a composition comprising a therapeutically effective amount of a nucleic acid or polypeptide. The terms "effective amount" and "therapeutically effective amount" each refer to the amount of a nucleic acid or polypeptide used to induce or enhance an effective immune response. It is preferred that compositions of the present invention provide for the induction or enhancement of an anti-tumor immune response in a host which protects the host from the development of a tumor and / or allows the host to eliminate an existing tumor from the body.

For oral administration, the pharmaceutical composition may be of any of several forms including, for example, a capsule, a tablet, a suspension, or liquid, among others. Liquids may be administered by injection as a composition with suitable carriers including saline, dextrose, or water. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intrasternal, infusion, or intraperitoneal administration. Suppositories for rectal administration of the drug can be prepared by mixing the drug with a suitable non-irritating excipient such as cocoa butter and polyethylene glycols that are solid at ordinary temperatures but liquid at the rectal temperature.

The dosage regimen for immunizing a host or otherwise treating a disorder or a disease with a composition of this invention is based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular compound employed. For example, a poxviral vector may be administered as a composition comprising 1×10^6 infectious particles per dose. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods.

A prime-boost regimen may also be utilized (WO 01/30382 A1) in which the targeted immunogen is initially administered in a priming step in one form followed by a boosting step in which the targeted immunogen is administered in another form. The form of the targeted immunogen in the priming and boosting steps are different. For instance, if the priming step utilized a nucleic acid, the boost may be administered as a peptide. Similarly, where a priming step utilized one type of recombinant virus (i.e., ALVAC), the boost step may utilize another type of virus (i.e., NYVAC). This prime-boost method of administration has been shown to induce strong immunological responses. Various combinations of forms are suitable in practicing the present invention.

While the compositions of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more other compositions or agents (i.e., other immunogenic targets, co-stimulatory molecules, adjuvants). When administered as a combination, the individual components can be formulated as separate compositions administered at the same time or different times, or the components can be combined as a single composition.

Injectable preparations, such as sterile injectable aqueous or oleaginous suspensions, may be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent. Suitable vehicles and solvents that may be employed are water, Ringer's solution, and isotonic sodium chloride solution, among others. For instance, a viral vector such as a poxvirus may be prepared in 0.4% NaCl. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed, including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

For topical administration, a suitable topical dose of a composition may be administered one to four, and preferably two or three times daily. The dose may also be administered with intervening days during which no does is applied. Suitable compositions may comprise from 0.001% to 10% w/w, for example, from 1% to 2% by weight of the formulation, although it may comprise as much as 10% w/w, but preferably not more than 5% w/w, and more preferably from 0.1% to 1% of the formulation. Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin (*e.g.*, liniments, lotions, ointments, creams, or pastes) and drops suitable for administration to the eye, ear, or nose.

The pharmaceutical compositions may also be prepared in a solid form (including granules, powders or suppositories). The pharmaceutical compositions may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc. Solid dosage forms for oral administration may include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose, or starch. Such dosage forms may also comprise, as in normal practice, additional substances other than inert diluents, *e.g.*, lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings. Liquid dosage forms for oral administration may include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, and elixirs containing inert diluents commonly used in the art, such as water. Such compositions may also comprise adjuvants, such as wetting sweetening, flavoring, and perfuming agents.

Pharmaceutical compositions comprising a nucleic acid or polypeptide of the present invention may take any of several forms and may be administered by any of several routes. In preferred embodiments, the compositions are administered via a parenteral route (intradermal, intramuscular or subcutaneous) to induce an immune response in the host. Alternatively, the composition may be administered directly into a lymph node (intranodal) or tumor mass (i.e., intratumoral administration). For example, the dose could be administered subcutaneously at days 0, 7, and 14. Suitable methods for immunization using compositions comprising TAs are known in the art, as shown for p53 (Hollstein et al., 1991), p21-ras (Almoguera et al., 1988), HER-2 (Fendly et.al., 1990), the melanoma-associated antigens (MAGE-1; MAGE-2) (van der

Bruggen et al., 1991), p97 (Hu et al., 1988), melanoma-associated antigen E (WO 99/30737) and carcinoembryonic antigen (CEA) (Kantor et al., 1993; Fishbein et al., 1992; Kaufman et al., 1991), among others.

Preferred embodiments of administratable compositions include, for example, nucleic acids or polypeptides in liquid preparations such as suspensions, syrups, or elixirs. Preferred injectable preparations include, for example, nucleic acids or polypeptides suitable for parental, subcutaneous, intradermal, intramuscular or intravenous administration such as sterile suspensions or emulsions. For example, a recombinant poxvirus may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The composition may also be provided in lyophilized form for reconstituting, for instance, in isotonic aqueous, saline buffer. In addition, the compositions can be co-administered or sequentially administered with other antineoplastic, anti-tumor or anti-cancer agents and/or with agents which reduce or alleviate ill effects of antineoplastic, anti-tumor or anti-cancer agents.

A kit comprising a composition of the present invention is also provided. The kit can include a separate container containing a suitable carrier, diluent or excipient. The kit can also include an additional anti-cancer, anti-tumor or antineoplastic agent and/or an agent that reduces or alleviates ill effects of antineoplastic, anti-tumor or anti-cancer agents for co- or sequential-administration. Additionally, the kit can include instructions for mixing or combining ingredients and/or administration.

A better understanding of the present invention and of its many advantages will be had from the following examples, given by way of illustration.

EXAMPLES

Example 1

Construction of the Multi-Antigen Construct vT416

The expression vector vT416 (ALVAC-NY-ESO-1/Trp-2-LFA-3/ICAM-1/B7.1-E3L/K3L) was constructed in the ALVAC vector using standard techniques. DNA sequences encoding NY-ESO-1, Trp-2, LFA-3, ICAM-1, B7.1, vvE3L and vvK3L were inserted into various loci within the ALVAC genome. DNA sequences encoding NY-ESO-1 (Chen et al. 1997 PNAS 94:1914) and TRP-2 (Wang et al. 1996 J. Exp. Med. 184:2207) were inserted into

the C5 locus. DNA sequences encoding LFA-3 (Wallner, et al. (1987) J. Exp. Med. 166:923-932), ICAM-1 (Staunton, et al. (1988) Cell 52:925-933) and B7.1 (Chen, et al. (1992) Cell 71:1093-1102) were inserted into the C3 locus. LFA-3, ICAM-1 and B7.1 form an expression cassette known as TRICOM. DNA sequences encoding vvE3L (Chang, et al. 1992. Proc. Natl. Acad. Sci. U. S. A 89:4825-4829) and vvK3L (Beattie, et al. 1991. Virology 183:419-422) were inserted into the C6 locus. Promoters were utilized as follows:

Table III

DNA sequence	Promoter
E3L	vaccinia E3L
K3L	vaccinia H6
LFA-3	vaccinia 30K
ICAM-1	vaccinia I3
B7.1	sE/L
NY-ESO-1	vaccinia H6
TRP-2	sE/L

Promoter sE/L is described by Chakrabarti, et al. (BioTechniques 23: 1094-1097, 1997).

The donor plasmids utilized are shown below:

Table IV

Plasmid	Size (bp)	Vector	Antibiotic Resitance Gene
pMPC6H6K3E3	-	pBS-SK	Amp
pALVAC.Tricom(C3) #33	10,470	pBS-SK	Amp
pT1132	11,154	pBS-SK	Amp

NY-ESO-1 and TRP-2 DNA sequences were inserted into the ALVAC donor plasmid pT1132. This donor plasmid was then used with pALVAC.Tricom(C3) #33 to generate the ALVAC-TRICOM recombinant expressing these genes using standard techniques. The plasmids pALVAC.Tricom(C3) #33 and pT1132 are shown in **Figure 1**. The DNA sequences of pALVAC.Tricom(C3) #33 and pT1132 are shown in **Figures 2 and 3**, respectively.

Example 2

Construction of the Multi-Antigen Construct vT419

The expression vector vT419 (ALVAC-gp100M/Mart-1/ Mage-1,3 minigene-LFA-3/ICAM-1/B7.1-E3L/K3L) was constructed in the ALVAC vector using standard techniques. DNA sequences encoding the gp100M/MART-1/MAGE-1,3 minigene, LFA-3, ICAM-1, B7.1, vvE3L and vvK3L were inserted into various loci within the ALVAC genome. The gp100M/ MART-1/MAGE-1,3 minigene was inserted into the C5 locus. DNA sequences encoding LFA-3 (Wallner, et al. (1987) J. Exp. Med. 166:923-932), ICAM-1 (Staunton, et al. (1988) Cell 52:925-933) and B7.1 (Chen, et al. (1992) Cell 71:1093-1102) were inserted into the C3 locus. LFA-3, ICAM-1 and B7.1 form an expression cassette known as TRICOM. DNA sequences encoding vvE3L (Chang, et al. 1992. Proc. Natl. Acad. Sci. U. S. A 89:4825-4829) and vvK3L (Beattie, et al. 1991. Virology 183:419-422) were inserted into the C6 locus. Promoters were utilized as follows:

Table V

Gene	Promoter
E3L	vaccinia E3L
K3L	vaccinia H6
LFA-3	vaccinia 30K
ICAM-1	vaccinia I3
B7.1	sE/L
gp100(M)	vaccinia H6
Mart-1	vaccinia 42K

Promoter sE/L is described by Chakrabarti, et al. (BioTechniques 23: 1094-1097, 1997).

The donor plasmids utilized are shown below:

Table VI

Plasmid	Size (bp)	Vector	Antibiotic Resitance Gene
PMPC6H6K3E3	-	pBS-SK	Amp
pALVAC.Tricom(C3) #33	10,470	pBS-SK	Amp
pT3217	11,465	pBS-SK	Amp

gp100(M), Mart-1 and Mage-1,3 minigene were inserted into the ALVAC C5 donor plasmid pT3217. This donor plasmid was then used with pALVAC.Tricom(C3) #33 to generate the ALVAC-TRICOM recombinant expressing these genes using standard techniques. This donor plasmid inserts into the C5 site. pALVAC.Tricom(C3) #33 is shown in **Figures 1** and **2.** The pT3217 plasmid is shown in **Figure 4**. The DNA sequence of pT3217 is shown in **Figure 5**.

EXAMPLE 3

Immunological Assessment of Multi-Antigen Vectors

The results of the first animal experiment indicated a trend toward higher immunological responses to three (Mart 1, NY-ESO-1 and gp100) of the four antigens when the vaccine was given as two separate injections. However, these differences were not statistically significant. In detail, HLA-A2/K^b transgenic mice (5/group) were immunized subcutaneously with vT419 (ALVAC(2)-gp100M/MART-1/MAGE-1/3 minigene/TRICOM) and vT416 (ALVAC(2)-TRP-2/NY-ESO-1/TRICOM) either combined at one site or given as separate injections. Control mice were immunized with parental ALVAC(2). Mice were vaccinated three times (at three week intervals), and three weeks after the last boost T cell responses in individual mice were analyzed by IFN-g ELISPOT and CTL assays following in vitro restimulation with peptide. Compared to control animals, mice vaccinated with the multi-antigen vectors (at 2 sites) exhibited statistically significant ELISPOT responses against MART-1. The IFN-gamma response to gp100M and NY-ESO-1 were also detectable, although these responses were not statistically significant due to response variability and the small number of cultures tested. ELISPOT responses against the TRP-2 antigen were elevated in all groups tested (including control animals), presumably due to the fact that the dominant A2-restricted TRP-2 peptide (180-188) cross-reacts with H-2K^b and can induce low avidity T cell responses in naïve mice following in vitro culture, and were therefore not statistically significant. Interestingly, ELISPOT responses in mice injected with an admixture of vT416 and vT419 were generally lower than in mice receiving each virus separately, although these differences did not achieve statistical significance. The CTL data were largely negative, except for one strong anti-gp100 response and one marginal anti-MART-1 response, both of which occurred in mice vaccinated with vT416 and vT419 (two sites). Overall, these results provided encouraging data that establish that the multi-antigen vectors can generate

responses against MART-1, and suggest that anti-gp100 and anti-NY-ESO-1 responses can also be induced.

Two additional pre-clinical animal studies have been completed using the melanoma multi-antigen ALVAC recombinants. In these experiments, HLA-A2/K^b transgenic mice (5/group) were immunized subcutaneously with vT419 (ALVAC(2)-gp100M/MART-1/MAGE-1/3 minigene/TRICOM) and vT416 (ALVAC(2)-TRP-2/NY-ESO-1/TRICOM) either combined at one site or given as separate injections. Control mice were immunized with parental ALVAC(2). After vaccination, the T cell responses in individual mice were assessed by IFN-gamma ELISPOT assay following in vitro restimulation with peptide. Unlike the previous multi-antigen experiment, which provided encouraging immunogenicity data, the two most recent studies generated inconclusive data, due to high background responses in control immunized animals. Therefore, overall the results were deemed as inconclusive.

To confirm the immunogenicity of the multi-antigen constructs, and to repeat results from the first study, another pre-clinical animal study has been completed. HLA-A2/K^b transgenic mice (10/group) were immunized subcutaneously with vT419 (ALVAC(2)-gp100M/MART-1/MAGE-1/3 minigene/TRICOM) and vT416 (ALVAC(2)-TRP-2/NY-ESO-1/TRICOM) given as separate injections. Control mice were immunized with parental ALVAC(2). Statistically significant ELISPOT responses were detectable against gp100, Mart-1 and TRP-2, and some responses were detected against NY-ESO-1, which were at the border of being statistically significant.

While the present invention has been described in terms of the preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations that come within the scope of the invention as claimed.

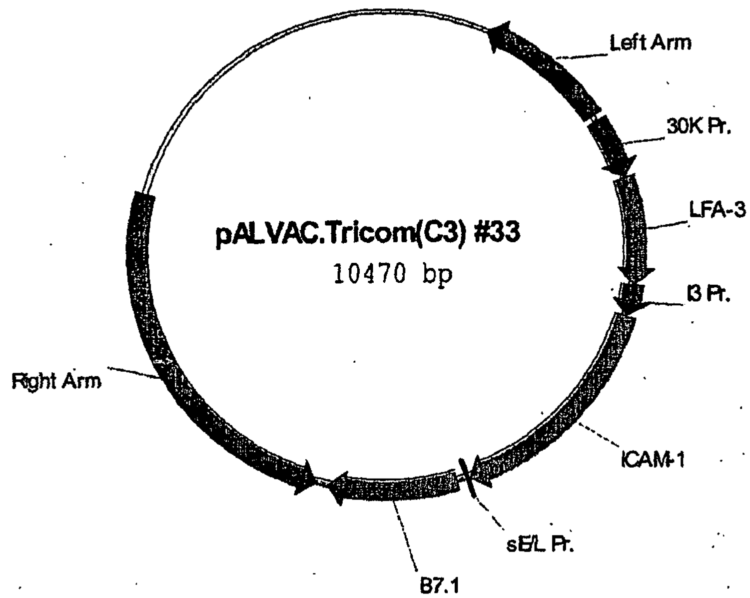
CLAIMS

What is claimed is:

1. An expression vector for co-expressing at least two immunogenic targets, wherein said immunogenic targets are selected from the group consisting of NY-ESO-1, TRP-2, gp100, gp100M, a MART antigen, MART-1, a MAGE antigen, MAGE-1, and MAGE-3.
2. The expression vector of claim 1 wherein the vector is a plasmid or a viral vector.
3. The expression vector of claim 2 wherein the viral vector is selected from the group consisting of poxvirus, adenovirus, retrovirus, herpesvirus, and adeno-associated virus.
4. The expression vector of claim 3 wherein the viral vector is a poxvirus selected from the group consisting of vaccinia, NYVAC, avipox, canarypox, ALVAC, ALVAC(2), fowlpox, and TROVAC.
5. The expression vector of claim 4 wherein the viral vector is a poxvirus selected from the group consisting of NYVAC, ALVAC, and ALVAC(2).
6. The expression vector of claim 1 further comprising at least one nucleic sequence encoding an angiogenesis-associated antigen.
7. The expression vector of claim 6 wherein the vector is a plasmid or a viral vector.
8. The expression vector of claim 7 wherein the viral vector is selected from the group consisting of poxvirus, adenovirus, retrovirus, herpesvirus, and adeno-associated virus.
9. The expression vector of claim 8 wherein the viral vector is a poxvirus selected from the group consisting of vaccinia, NYVAC, avipox, canarypox, ALVAC, ALVAC(2), fowlpox, and TROVAC.
10. The expression vector of claim 9 wherein the viral vector is a poxvirus selected from the group consisting of NYVAC, ALVAC, and ALVAC(2).
11. The expression vector of claim 1 or 6 further comprising at least one nucleic acid sequence encoding a co-stimulatory component.
12. The expression vector of claim 11 wherein the vector is a plasmid or a viral vector.
13. The expression vector of claim 12 wherein the viral vector is selected from the group consisting of poxvirus, adenovirus, retrovirus, herpesvirus, and adeno-associated virus.
14. The expression vector of claim 13 wherein the viral vector is a poxvirus selected from the group consisting of vaccinia, NYVAC, avipox, canarypox, ALVAC, ALVAC(2), fowlpox, and TROVAC.

15. The expression vector of claim 14 wherein the viral vector is a poxvirus selected from the group consisting of NYVAC, ALVAC, and ALVAC(2).
16. The expression vector of any one claims 11-15 wherein the co-stimulatory component is human B7.1.
17. A composition comprising an expression vector of any one of claims 1-16 in a pharmaceutically acceptable carrier.
18. A method for preventing or treating cancer comprising administering to a host an expression vector of any one of claims 1-16.
19. A method for preventing or treating cancer comprising administering to a host a composition of claim 17.

FIGURE 1


Left Arm
30K Pr.
LFA-3
I3 Pr.
ICAM-1
sE/L Pr.
B7.1
Right Arm
pALVAC.Tricom(C3) #33
10470 bp

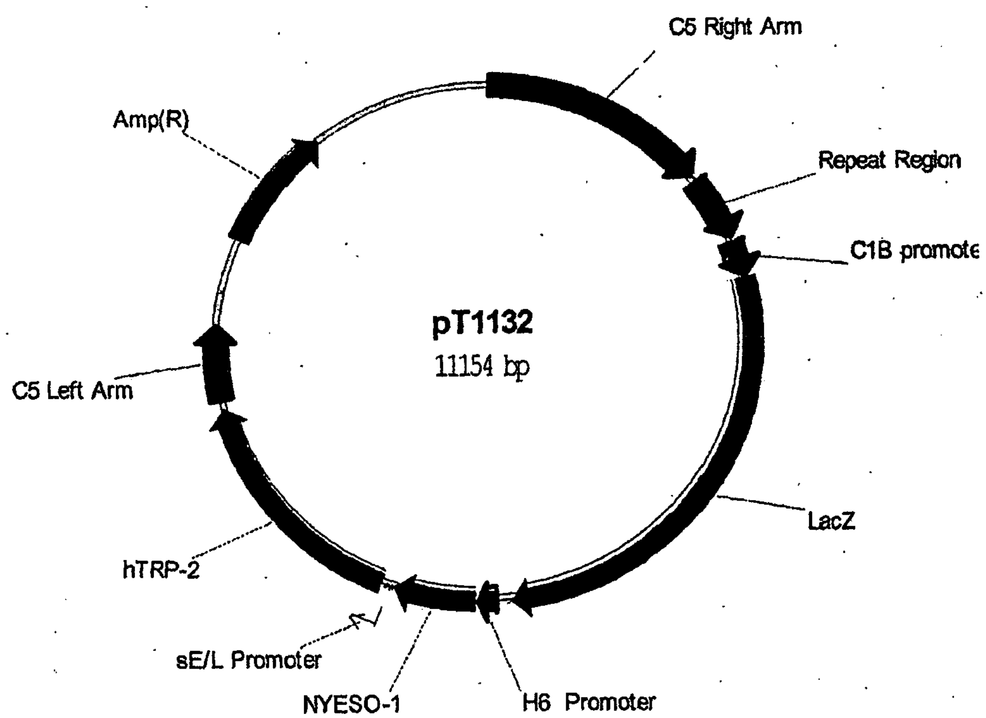

C5 Right Arm
Repeat Region
C1B promote
LacZ
H6 Promoter
NYESO-1
sE/L Promoter
hTRP-2
C5 Left Arm
Amp(R)
pT1132
11154 bp

FIGURE 2

DNA Sequence of pALVAC.Tricom(C3) #33

```
  1  GGAAATTGTA AACGTTAATA TTTTGTTAAA ATTCGCGTTA AATTTTTGTT
     CCTTTAACAT TTGCAATTAT AAAACAATTT TAAGCGCAAT TTAAAAACAA
 51  AAATCAGCTC ATTTTTTAAC CAATAGGCCG AAATCGGCAA AATCCCTTAT
     TTTAGTCGAG TAAAAAATTG GTTATCCGGC TTTAGCCGTT TTAGGGAATA
101  AAATCAAAAG AATAGACCGA GATAGGGTTG AGTGTTGTTC CAGTTTGGAA
     TTTAGTTTTC TTATCTGGCT CTATCCCAAC TCACAACAAG GTCAAACCTT
151  CAAGAGTCCA CTATTAAAGA ACGTGGACTC CAACGTCAAA GGGCGAAAAA
     GTTCTCAGGT GATAATTTCT TGCACCTGAG GTTGCAGTTT CCCGCTTTTT
201  CCGTCTATCA GGGCGATGGC CCACTACGTG AACCATCACC CTAATCAAGT
     GGCAGATAGT CCCGCTACCG GGTGATGCAC TTGGTAGTGG GATTAGTTCA
251  TTTTTGGGGT CGAGGTGCCG TAAAGCACTA AATCGGAACC CTAAAGGGAG
     AAAAACCCCA GCTCCACGGC ATTTCGTGAT TTAGCCTTGG GATTTCCCTC
301  CCCCCGATTT AGAGCTTGAC GGGGAAAGCC GGCGAACGTG GCGAGAAAGG
     GGGGGCTAAA TCTCGAACTG CCCCTTTCGG CCGCTTGCAC CGCTCTTTCC
351  AAGGGAAGAA AGCGAAAGGA GCGGGCGCTA GGGCGCTGGC AAGTGTAGCG
     TTCCCTTCTT TCGCTTTCCT CGCCCGCGAT CCCGCGACCG TTCACATCGC
401  GTCACGCTGC GCGTAACCAC CACACCCGCC GCGCTTAATG CGCCGCTACA
     CAGTGCGACG CGCATTGGTG GTGTGGGCGG CGCGAATTAC GCGGCGATGT
451  GGGCGCGTCG CGCCATTCGC CATTCAGGCT GCGCAACTGT TGGGAAGGGC
     CCCGCGCAGC GCGGTAAGCG GTAAGTCCGA CGCGTTGACA ACCCTTCCCG
501  GATCGGTGCG GGCCTCTTCG CTATTACGCC AGCTGGCGAA AGGGGGATGT
     CTAGCCACGC CCGGAGAAGC GATAATGCGG TCGACCGCTT TCCCCCTACA
551  GCTGCAAGGC GATTAAGTTG GGTAACGCCA GGGTTTTCCC AGTCACGACG
     CGACGTTCCG CTAATTCAAC CCATTGCGGT CCCAAAAGGG TCAGTGCTGC
601  TTGTAAAACG ACGGCCAGTG AATTGTAATA CGACTCACTA TAGGGCGAAT
     AACATTTTGC TGCCGGTCAC TTAACATTAT GCTGAGTGAT ATCCCGCTTA
651  TGGGTACCGC GGCCGCGTCG ACATGCATTG TTAGTTCTGT AGATCAGTAA
     ACCCATGGCG CCGGCGCAGC TGTACGTAAC AATCAAGACA TCTAGTCATT
                       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                                  Left Arm
701  CGTATAGCAT ACGAGTATAA TTATCGTAGG TAGTAGGTAT CCTAAAATAA
     GCATATCGTA TGCTCATATT AATAGCATCC ATCATCCATA GGATTTTATT
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          Left Arm
751  ATCTGATACA GATAATAACT TTGTAAATCA ATTCAGCAAT TTCTCTATTA
     TAGACTATGT CTATTATTGA AACATTTAGT TAAGTCGTTA AAGAGATAAT
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          Left Arm
801  TCATGATAAT GATTAATACA CAGCGTGTCG TTATTTTTTG TTACGATAGT
     AGTACTATTA CTAATTATGT GTCGCACAGC AATAAAAAAC AATGCTATCA
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          Left Arm
851  ATTTCTAAAG TAAAGAGCAG GAATCCCTAG TATAATAGAA ATAATCCATA
     TAAAGATTTC ATTTCTCGTC CTTAGGGATC ATATTATCTT TATTAGGTAT
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          Left Arm
901  TGAAAAATAT AGTAATGTAC ATATTTCTAA TGTTAACATA TTTATAGGTA
     ACTTTTTATA TCATTACATG TATAAAGATT ACAATTGTAT AAATATCCAT
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                          Left Arm
951  AATCCAGGAA GGGTAATTTT TACATATCTA TATACGCTTA TTACAGTTAT
     TTAGGTCCTT CCCATTAAAA ATGTATAGAT ATATGCGAAT AATGTCAATA
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Left Arm
1001 TAAAAATATA CTTGCAAACA TGTTAGAAGT AAAAAAGAAA GAACTAATTT
ATTTTTATAT GAACGTTTGT ACAATCTTCA TTTTTTCTTT CTTGATTAAA
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Left Arm
1051 TACAAAGTGC TTTACCAAAA TGCCAATGGA AATTACTTAG TATGTATATA
ATGTTTCACG AAATGGTTTT ACGGTTACCT TTAATGAATC ATACATATAT
~~~~~~
Left Arm
1101 ATGTATAAAG GTATGAATAT CACAAACAGC AAATCGGCTA TTCCCAAGTT
TACATATTTC CATACTTATA GTGTTTGTCG TTTAGCCGAT AAGGGTTCAA
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Left Arm

1151 GAGAAACGGT ATAATAGATA TATTTCTAGA TACCATTAAT AACCTTATAA
CTCTTTGCCA TATTATCTAT ATAAAGATCT ATGGTAATTA TTGGAATATT
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Left Arm
1201 GCTTGACGTT TCCTATAATG CCTACTAAGA AAACTAGAAG ATACATACAT
CGAACTGCAA AGGATATTAC GGATGATTCT TTTGATCTTC TATGTATGTA
~~~~~~
Left Arm
1251 ACTAACGCCA TACGAGAGTA ACTACTCATC GTATAACTAC TGTTGCTAAC
TGATTGCGGT ATGCTCTCAT TGATGAGTAG CATATTGATG ACAACGATTG
~~~~~~
Left Arm
1301 AGTGACACTG ATGTTATAAC TCATCTTTGA TGTGGTATAA ATGTATAATA
TCACTGTGAC TACAATATTG AGTAGAAACT ACACCATATT TACATATTAT
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Left Arm
1351 ACTATATTAC ACTGGTATTT TATTTCAGTT ATATACTATA TAGTATTAAA
TGATATAATG TGACCATAAA ATAAAGTCAA TATATGATAT ATCATAATTT
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Left Arm
1401 AATTATATTT GTATAATTAT ATTATTATAT TCAGTGTAGA AAGTAAAATA
TTAATATAAA CATATTAATA TAATAATATA AGTCACATCT TTCATTTTAT
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Left Arm
1451 CTATAAATAT GTATCTCTTA TTTATAACTT ATTAGTAAAG TATGTACTAT
GATATTTATA CATAGAGAAT AAATATTGAA TAATCATTTC ATACATGATA
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Left Arm
1501 TCAGTTATAT TGTTTTATAA AAGCTAAATG CTACTAGATT GATATAAATG
AGTCAATATA ACAAAATATT TTCGATTTAC GATGATCTAA CTATATTTAC
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Left Arm
1551 AATATGTAAT AAATTAGTAA TGTAGTATAC TAATATTAAC TCACATTTGA
TTATACATTA TTTAATCATT ACATCATATG ATTATAATTG AGTGTAAACT
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Left Arm
30K Pr.
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1601 CTAATTAGCT ATAAAAACCC TAAGGTAGGC GGCCGCACTA GAGGATTCGA
GATTAATCGA TATTTTTGGG ATTCCATCCG CCGGCGTGAT CTCCTAAGCT

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                         30K Pr.
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1651  CAAACACCAA TAATTCCCTT CTCTTCATTC CGGACATTAA ATTGGCTATA
      GTTTGTGGTT ATTAAGGGAA GAGAAGTAAG GCCTGTAATT TAACCGATAT
                         30K Pr.
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1701  GATAATAAAG ACATTGAGAT GTTACAGGCT CTGTTCAAAT ACGACATTAA
      CTATTATTTC TGTAACTCTA CAATGTCCGA GACAAGTTTA TGCTGTAATT
                         30K Pr.
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1751  TATCTATTCT GCTAATCTGG AAAATGTACT ATTGGATGAT GCCGAAATAG
      ATAGATAAGA CGATTAGACC TTTTACATGA TAACCTACTA CGGCTTTATC
                         30K Pr.
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1801  CTAAAATGAT TATAGAAAAG CATGTTGAAT ACAAGTCTGA CTCCTATACA
      GATTTTACTA ATATCTTTTC GTACAACTTA TGTTCAGACT GAGGATATGT
                         30K Pr.
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1851  AAAGATCTCG ATATAGTCAA GAATAATAAA TTGGATGAAA TAATTAGCAA
      TTTCTAGAGC TATATCAGTT CTTATTATTT AACCTACTTT ATTAATCGTT
                         30K Pr.
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1901  AAACAAGGAA CTCAGACTCA TGTACGTCAA TTGTGTAAAG AAAAACTAAT
      TTTGTTCCTT GAGTCTGAGT ACATGCAGTT AACACATTTC TTTTTGATTA
                         30K Pr.
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1951  TAGATTCTCC CACATTTTTG TTAACATTAC ACTAACTAAT TGGTAAAATT
      ATCTAAGAGG GTGTAAAAAC AATTGTAATG TGATTGATTA ACCATTTTAA
                         30K Pr.
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2001  GATAGAATAA TTATGTGTAT ATAAGATAGA TTTCCTATTG TCTTACTCAT
      CTATCTTATT AATACACATA TATTCTATCT AAAGGATAAC AGAATGAGTA
                 30K Pr.
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                                                 hLFA-3
                                            ~~~~~~~~~~~~~~~~~~~
2051  TGCATCGTGG GAATTCAGAT CAGCTTCCGC GGCATGGTTG CTGGGAGCGA
      ACGTAGCACC CTTAAGTCTA GTCGAAGGCG CCGTACCAAC GACCCTCGCT
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                      hLFA-3
2101  CGCGGGGCGG GCCCTGGGGG TCCTCAGCGT GGTCTGCCTG CTGCACTGCT
      GCGCCCCGCC CGGGACCCCC AGGAGTCGCA CCAGACGGAC GACGTGACGA
                      hLFA-3
2151  TTGGTTTCAT CAGCTGTTTT TCCCAACAAA TATATGGTGT TGTGTATGGG
      AACCAAAGTA GTCGACAAAA AGGGTTGTTT ATATACCACA ACACATACCC
                      hLFA-3
2201  AATGTAACTT TCCATGTACC AAGCAATGTG CCTTTAAAAG AGGTCCTATG
      TTACATTGAA AGGTACATGG TTCGTTACAC GGAAATTTTC TCCAGGATAC
                      hLFA-3
2251  GAAAAAACAA AAGGATAAAG TTGCAGAACT GGAAAATTCT GAATTCAGAG
      CTTTTTTGTT TTCCTATTTC AACGTCTTGA CCTTTTAAGA CTTAAGTCTC
                      hLFA-3
2301  CTTTCTCATC TTTTAAAAAT AGGGTTTATT TAGACACTGT GTCAGGTAGC
      GAAAGAGTAG AAAATTTTTA TCCCAAATAA ATCTGTGACA CAGTCCATCG
                      hLFA-3
2351  CTCACTATCT ACAACTTAAC ATCATCAGAT GAAGATGAGT ATGAAATGGA
      GAGTGATAGA TGTTGAATTG TAGTAGTCTA CTTCTACTCA TACTTTACCT
                      hLFA-3
2401  ATCGCCAAAT ATTACTGATA CCATGAAGTT CTTTCTTTAT GTGCTTGAGT
      TAGCGGTTTA TAATGACTAT GGTACTTCAA GAAAGAAATA CACGAACTCA
                      hLFA-3
2451  CTCTTCCATC TCCCACACTA ACTTGTGCAT TGACTAATGG AAGCATTGAA
      GAGAAGGTAG AGGGTGTGAT TGAACACGTA ACTGATTACC TTCGTAACTT
                      hLFA-3
2501  GTCCAATGCA TGATACCAGA GCATTACAAC AGCCATCGAG GACTTATAAT
      CAGGTTACGT ACTATGGTCT CGTAATGTTG TCGGTAGCTC CTGAATATTA
                      hLFA-3
2551  GTACTCATGG GATTGTCCTA TGGAGCAATG TAAACGTAAC TCAACCAGTA
      CATGAGTACC CTAACAGGAT ACCTCGTTAC ATTTGCATTG AGTTGGTCAT
                      hLFA-3
2601  TATATTTTAA GATGGAAAAT GATCTTCCAC AAAAAATACA GTGTACTCTT
      ATATAAAATT CTACCTTTTA CTAGAAGGTG TTTTTTATGT CACATGAGAA
                      hLFA-3
2651  AGCAATCCAT TATTTAATAC AACATCATCA ATCATTTTGA CAACCTGTAT
      TCGTTAGGTA ATAAATTATG TTGTAGTAGT TAGTAAAACT GTTGGACATA
                      hLFA-3
2701  CCCAAGCAGC GGTCATTCAA GACACAGATA TGCACTTATA CCCATACCAT
      GGGTTCGTCG CCAGTAAGTT CTGTGTCTAT ACGTGAATAT GGGTATGGTA
                      hLFA-3
2751  TAGCAGTAAT TACAACATGT ATTGTGCTGT ATATGAATGG TATTCTGAAA
      ATCGTCATTA ATGTTGTACA TAACACGACA TATACTTACC ATAAGACTTT
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                          hLFA-3                                      I3 Pr.
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2801      TGTGACAGAA AACCAGACAG AACCAACTCC AATTGATTGG CTCGACCGGG
          ACACTGTCTT TTGGTCTGTC TTGGTTGAGG TTAACTAACC GAGCTGGCCC
                                I3 Pr.
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2851      AATGTACTAT CTACGTACGA AACCCGCATC CGCTCCCATT CAATTCACAT
          TTACATGATA GATGCATGCT TTGGGCGTAG GCGAGGGTAA GTTAAGTGTA
                                I3 Pr.
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2901      TGGACAAGGA TAAAATAAAA CCACTGGTGG TTTGCGATTC CGAAATCTGT
          ACCTGTTCCT ATTTTATTTT GGTGACCACC AAACGCTAAG GCTTTAGACA
                                I3 Pr.
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2951      ACATCATGCA GTGGTTAAAC AAAAACATTT TTATTCTCAA ATGAGATAAA
          TGTAGTACGT CACCAATTTG TTTTTGTAAA AATAAGAGTT TACTCTATTT
                                I3 Pr.
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3001      GTGAAAATAT ATATCATTAT ATTACAAAGT ACAATTATTT AGGTTTAATC
          CACTTTTATA TATAGTAATA TAATGTTTCA TGTTAATAAA TCCAAATTAG
            I3 Pr.                           hICAM
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3051      AATCCCGCGG GCTATGGCTC CCAGCAGCCC CCGGCCCGCG CTGCCCGCAC
          TTAGGGCGCC CGATACCGAG GGTCGTCGGG GGCCGGGCGC GACGGGCGTG
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3101      TCCTGGTCCT GCTCGGGGCT CTGTTCCCAG GACCTGGCAA TGCCCAGACA
          AGGACCAGGA CGAGCCCCGA GACAAGGGTC CTGGACCGTT ACGGGTCTGT
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3151      TCTGTGTCCC CCTCAAAAGT CATCCTGCCC CGGGGAGGCT CCGTGCTGGT
          AGACACAGGG GGAGTTTTCA GTAGGACGGG GCCCCTCCGA GGCACGACCA
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3201      GACATGCAGC ACCTCCTGTG ACCAGCCCAA GTTGTTGGGC ATAGAGACCC
          CTGTACGTCG TGGAGGACAC TGGTCGGGTT CAACAACCCG TATCTCTGGG
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3251      CGTTGCCTAA AAAGGAGTTG CTCCTGCCTG GGAACAACCG GAAGGTGTAT
          GCAACGGATT TTTCCTCAAC GAGGACGGAC CCTTGTTGGC CTTCCACATA
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3301      GAACTGAGCA ATGTGCAAGA AGATAGCCAA CCAATGTGCT ATTCAAACTG
          CTTGACTCGT TACACGTTCT TCTATCGGTT GGTTACACGA TAAGTTTGAC
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3351      CCCTGATGGG CAGTCAACAG CTAAAACCTT CCTCACCGTG TACTGGACTC
          GGGACTACCC GTCAGTTGTC GATTTTGGAA GGAGTGGCAC ATGACCTGAG
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3401      CAGAACGGGT GGAACTGGCA CCCCTCCCCT CTTGGCAGCC AGTGGGCAAG
          GTCTTGCCCA CCTTGACCGT GGGGAGGGGA GAACCGTCGG TCACCCGTTC
                                hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3451      AACCTTACCC TACGCTGCCA GGTGGAGGGT GGGGCACCCC GGGCCAACCT
          TTGGAATGGG ATGCGACGGT CCACCTCCCA CCCCGTGGGG CCCGGTTGGA
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                                    hICAM
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     3501  CACCGTGGTG CTGCTCCGTG GGGAGAAGGA GCTGAAACGG GAGCCAGCTG
           GTGGCACCAC GACGAGGCAC CCCTCTTCCT CGACTTTGCC CTCGGTCGAC
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3551  TGGGGGAGCC CGCTGAGGTC ACGACCACGG TGCTGGTGAG GAGAGATCAC
           ACCCCCTCGG GCGACTCCAG TGCTGGTGCC ACGACCACTC CTCTCTAGTG
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3601  CATGGAGCCA ATTTCTCGTG CCGCACTGAA CTGGACCTGC GGCCCCAAGG
           GTACCTCGGT TAAAGAGCAC GGCGTGACTT GACCTGGACG CCGGGGTTCC
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3651  GCTGGAGCTG TTTGAGAACA CCTCGGCCCC CTACCAGCTC CAGACCTTTG
           CGACCTCGAC AAACTCTTGT GGAGCCGGGG GATGGTCGAG GTCTGGAAAC
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3701  TCCTGCCAGC GACTCCCCCA CAACTTGTCA GCCCCCGGGT CCTAGAGGTG
           AGGACGGTCG CTGAGGGGGT GTTGAACAGT CGGGGGCCCA GGATCTCCAC
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3751  GACACGCAGG GGACCGTGGT CTGTTCCCTG GACGGGCTGT TCCCAGTCTC
           CTGTGCGTCC CCTGGCACCA GACAAGGGAC CTGCCCGACA AGGGTCAGAG
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3801  GGAGGCCCAG GTCCACCTGG CACTGGGGGA CCAGAGGTTG AACCCCACAG
           CCTCCGGGTC CAGGTGGACC GTGACCCCCT GGTCTCCAAC TTGGGGTGTC
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3851  TCACCTATGG CAACGACTCC TTCTCGGCCA AGGCCTCAGT CAGTGTGACC
           AGTGGATACC GTTGCTGAGG AAGAGCCGGT TCCGGAGTCA GTCACACTGG
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3901  GCAGAGGACG AGGGCACCCA GCGGCTGACG TGTGCAGTAA TACTGGGGAA
           CGTCTCCTGC TCCCGTGGGT CGCCGACTGC ACACGTCATT ATGACCCCTT
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     3951  CCAGAGCCAG GAGACACTGC AGACAGTGAC CATCTACAGC TTTCCGGCGC
           GGTCTCGGTC CTCTGTGACG TCTGTCACTG GTAGATGTCG AAAGGCCGCG
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     4001  CCAACGTGAT TCTGACGAAG CCAGAGGTCT CAGAAGGGAC CGAGGTGACA
           GGTTGCACTA AGACTGCTTC GGTCTCCAGA GTCTTCCCTG GCTCCACTGT
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     4051  GTGAAGTGTG AGGCCCACCC TAGAGCCAAG GTGACGCTGA ATGGGGTTCC
           CACTTCACAC TCCGGGTGGG ATCTCGGTTC CACTGCGACT TACCCCAAGG
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     4101  AGCCCAGCCA CTGGGCCCGA GGGCCCAGCT CCTGCTGAAG GCCACCCCAG
           TCGGGTCGGT GACCCGGGCT CCCGGGTCGA GGACGACTTC CGGTGGGGTC
                                    hICAM
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     4151  AGGACAACGG GCGCAGCTTC TCCTGCTCTG CAACCCTGGA GGTGGCCGGC
           TCCTGTTGCC CGCGTCGAAG AGGACGAGAC GTTGGGACCT CCACCGGCCG
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                           hICAM
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4201      CAGCTTATAC ACAAGAACCA GACCCGGGAG CTTCGTGTCC TGTATGGCCC
          GTCGAATATG TGTTCTTGGT CTGGGCCCTC GAAGCACAGG ACATACCGGG
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4251      CCGACTGGAC GAGAGGGATT GTCCGGGAAA CTGGACGTGG CCAGAAAATT
          GGCTGACCTG CTCTCCCTAA CAGGCCCTTT GACCTGCACC GGTCTTTTAA
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4301      CCCAGCAGAC TCCAATGTGC CAGGCTTGGG GGAACCCATT GCCCGAGCTC
          GGGTCGTCTG AGGTTACACG GTCCGAACCC CCTTGGGTAA CGGGCTCGAG
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4351      AAGTGTCTAA AGGATGGCAC TTTCCCACTG CCCATCGGGG AATCAGTGAC
          TTCACAGATT TCCTACCGTG AAAGGGTGAC GGGTAGCCCC TTAGTCACTG
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4401      TGTCACTCGA GATCTTGAGG GCACCTACCT CTGTCGGGCC AGGAGCACTC
          ACAGTGAGCT CTAGAACTCC CGTGGATGGA GACAGCCCGG TCCTCGTGAG
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4451      AAGGGGAGGT CACCCGCGAG GTGACCGTGA ATGTGCTCTC CCCCCGGTAT
          TTCCCCTCCA GTGGGCGCTC CACTGGCACT TACACGAGAG GGGGGCCATA
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4501      GAGATTGTCA TCATCACTGT GGTAGCAGCC GCAGTCATAA TGGGCACTGC
          CTCTAACAGT AGTAGTGACA CCATCGTCGG CGTCAGTATT ACCCGTGACG
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4551      AGGCCTCAGC ACGTACCTCT ATAACCGCCA GCGGAAGATC AAGAAATACA
          TCCGGAGTCG TGCATGGAGA TATTGGCGGT CGCCTTCTAG TTCTTTATGT
                           hICAM
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4601      GACTACAACA GGCCCAAAAA GGGACCCCCA TGAAACCGAA CACACAAGCC
          CTGATGTTGT CCGGGTTTTT CCCTGGGGGT ACTTTGGCTT GTGTGTTCGG
             hICAM                              sE/L Pr.
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4651      ACGCCTCCCT GAGCATGCAT GTAGCTTAAA AATTGAAATT TTATTTTTTT
          TGCGGAGGGA CTCGTACGTA CATCGAATTT TTAACTTTAA AATAAAAAAA
              sE/L Pr.
          ~~~~~~~~~~~~~~~~~
4701      TTTTTGGAAT ATAAATAAGC TCGAAGTCGA AATTCCTGCA GCCCGGGGCC
          AAAAACCTTA TATTTATTCG AGCTTCAGCT TTAAGGACGT CGGGCCCCGG
                           hB7.1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4751      ATGGGCCACA CACGGAGGCA GGGAACATCA CCATCCAAGT GTCCATACCT
          TACCCGGTGT GTGCCTCCGT CCCTTGTAGT GGTAGGTTCA CAGGTATGGA
                           hB7.1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4801      CAATTTCTTT CAGCTCTTGG TGCTGGCTGG TCTTTCTCAC TTCTGTTCAG
          GTTAAAGAAA GTCGAGAACC ACGACCGACC AGAAAGAGTG AAGACAAGTC
                           hB7.1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4851      GTGTTATCCA CGTGACCAAG GAAGTGAAAG AAGTGGCAAC GCTGTCCTGT
          CACAATAGGT GCACTGGTTC CTTCACTTTC TTCACCGTTG CGACAGGACA
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                                  hB7.1
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   4901  GGTCACAATG TTTCTGTTGA AGAGCTGGCA CAAACTCGCA TCTACTGGCA
         CCAGTGTTAC AAAGACAACT TCTCGACCGT GTTTGAGCGT AGATGACCGT
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   4951  AAAGGAGAAG AAAATGGTGC TGACTATGAT GTCTGGAGAC ATGAATATAT
         TTTCCTCTTC TTTTACCACG ACTGATACTA CAGACCTCTG TACTTATATA
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5001  GGCCCGAGTA CAAGAACCGG ACCATCTTTG ATATCACTAA TAACCTCTCC
         CCGGGCTCAT GTTCTTGGCC TGGTAGAAAC TATAGTGATT ATTGGAGAGG
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5051  ATTGTGATCC TGGCTCTGCG CCCATCTGAC GAGGGCACAT ACGAGTGTGT
         TAACACTAGG ACCGAGACGC GGGTAGACTG CTCCCGTGTA TGCTCACACA
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5101  TGTTCTGAAG TATGAAAAAG ACGCTTTCAA GCGGGAACAC CTGGCTGAAG
         ACAAGACTTC ATACTTTTTC TGCGAAAGTT CGCCCTTGTG GACCGACTTC
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5151  TGACGTTATC AGTCAAAGCT GACTTCCCTA CACCTAGTAT ATCTGACTTT
         ACTGCAATAG TCAGTTTCGA CTGAAGGGAT GTGGATCATA TAGACTGAAA
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5201  GAAATTCCAA CTTCTAATAT TAGAAGGATA ATTTGCTCAA CCTCTGGAGG
         CTTTAAGGTT GAAGATTATA ATCTTCCTAT TAAACGAGTT GGAGACCTCC
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5251  TTTTCCAGAG CCTCACCTCT CCTGGTTGGA AAATGGAGAA GAATTAAATG
         AAAAGGTCTC GGAGTGGAGA GGACCAACCT TTTACCTCTT CTTAATTTAC
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5301  CCATCAACAC AACAGTTTCC CAAGATCCTG AAACTGAGCT CTATGCTGTT
         GGTAGTTGTG TTGTCAAAGG GTTCTAGGAC TTTGACTCGA GATACGACAA
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5351  AGCAGCAAAC TGGATTTCAA TATGACAACC AACCACAGCT TCATGTGTCT
         TCGTCGTTTG ACCTAAAGTT ATACTGTTGG TTGGTGTCGA AGTACACAGA
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5401  CATCAAGTAT GGACATTTAA GAGTGAATCA GACCTTCAAC TGGAATACAA
         GTAGTTCATA CCTGTAAATT CTCACTTAGT CTGGAAGTTG ACCTTATGTT
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5451  CCAAGCAAGA GCATTTTCCT GATAACCTGC TCCCATCCTG GGCCATTACC
         GGTTCGTTCT CGTAAAAGGA CTATTGGACG AGGGTAGGAC CCGGTAATGG
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5501  TTAATCTCAG TAAATGGAAT TTTCGTGATA TGCTGCCTGA CCTACTGCTT
         AATTAGAGTC ATTTACCTTA AAAGCACTAT ACGACGGACT GGATGACGAA
                                  hB7.1
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   5551  TGCCCCACGC TGCAGAGAGA GAAGGAGGAA TGAGAGATTG AGAAGGGAAA
         ACGGGGTGCG ACGTCTCTCT CTTCCTCCTT ACTCTCTAAC TCTTCCCTTT
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          hB7.1
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5601      GTGTACGCCC TGTATAAAAG CTTTCTAGGT TTTTGTTTAG GGCTGCAGGA
          CACATGCGGG ACATATTTTC GAAAGATCCA AAAACAAATC CCGACGTCCT
5651      ATTCCTCGAG GGATCCCGAT TTTTATGACT AGTTAATCAA ATAAAAAGCA
          TAAGGAGCTC CCTAGGGCTA AAAATACTGA TCAATTAGTT TATTTTTCGT
                                                          Right Arm
5701      TACAAGCTAT TGCTTCGCTA TCGTTACAAA ATGGCAGGAA TTTTGTGTAA
          ATGTTCGATA ACGAAGCGAT AGCAATGTTT TACCGTCCTT AAAACACATT
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
5751      ACTAAGCCAC ATACTTGCCA ATGAAAAAAA TAGTAGAAAG GATACTATTT
          TGATTCGGTG TATGAACGGT TACTTTTTTT ATCATCTTTC CTATGATAAA
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
5801      TAATGGGATT AGATGTTAAG GTTCCTTGGG ATTATAGTAA CTGGGCATCT
          ATTACCCTAA TCTACAATTC CAAGGAACCC TAATATCATT GACCCGTAGA
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
5851      GTTAACTTTT ACGACGTTAG GTTAGATACT GATGTTACAG ATTATAATAA
          CAATTGAAAA TGCTGCAATC CAATCTATGA CTACAATGTC TAATATTATT
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
5901      TGTTACAATA AAATACATGA CAGGATGTGA TATTTTTCCT CATATAACTC
          ACAATGTTAT TTTATGTACT GTCCTACACT ATAAAAAGGA GTATATTGAG
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
5951      TTGGAATAGC AAATATGGAT CAATGTGATA GATTTGAAAA TTTCAAAAAG
          AACCTTATCG TTTATACCTA GTTACACTAT CTAAACTTTT AAAGTTTTTC
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
6001      CAAATAACTG ATCAAGATTT ACAGACTATT TCTATAGTCT GTAAAGAAGA
          GTTTATTGAC TAGTTCTAAA TGTCTGATAA AGATATCAGA CATTTCTTCT
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
6051      GATGTGTTTT CCTCAGAGTA ACGCCTCTAA ACAGTTGGGA GCGAAAGGAT
          CTACACAAAA GGAGTCTCAT TGCGGAGATT TGTCAACCCT CGCTTTCCTA
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
6101      GCGCTGTAGT TATGAAACTG GAGGTATCTG ATGAACTTAG AGCCCTAAGA
          CGCGACATCA ATACTTTGAC CTCCATAGAC TACTTGAATC TCGGGATTCT
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
6151      AATGTTCTGC TGAATGCGGT ACCCTGTTCG AAGGACGTGT TTGGTGATAT
          TTACAAGACG ACTTACGCCA TGGGACAAGC TTCCTGCACA AACCACTATA
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
6201      CACAGTAGAT AATCCGTGGA ATCCTCACAT AACAGTAGGA TATGTTAAGG
          GTGTCATCTA TTAGGCACCT TAGGAGTGTA TTGTCATCCT ATACAATTCC
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
6251      AGGACGATGT CGAAAACAAG AAACGCCTAA TGGAGTGCAT GTCCAAGTTT
          TCCTGCTACA GCTTTTGTTC TTTGCGGATT ACCTCACGTA CAGGTTCAAA
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                              Right Arm
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6301   AGGGGGCAAG AAATACAAGT TCTAGGATGG TATTAATAAG TATCTAAGTA
       TCCCCCGTTC TTTATGTTCA AGATCCTACC ATAATTATTC ATAGATTCAT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6351   TTTGGTATAA TTTATTAAAT AGTATAATTA TAACAAATAA TAAATAACAT
       AAACCATATT AAATAATTTA TCATATTAAT ATTGTTTATT ATTTATTGTA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6401   GATAACGGTT TTTATTAGAA TAAAATAGAG ATAATATCAT AATGATATAT
       CTATTGCCAA AAATAATCTT ATTTTATCTC TATTATAGTA TTACTATATA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6451   AATACTTCAT TACCAGAAAT GAGTAATGGA AGACTTATAA ATGAACTGCA
       TTATGAAGTA ATGGTCTTTA CTCATTACCT TCTGAATATT TACTTGACGT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6501   TAAAGCTATA AGGTATAGAG ATATAAATTT AGTAAGGTAT ATACTTAAAA
       ATTTCGATAT TCCATATCTC TATATTTAAA TCATTCCATA TATGAATTTT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6551   AATGCAAATA CAATAACGTA AATATACTAT CAACGTCTTT GTATTTAGCC
       TTACGTTTAT GTTATTGCAT TTATATGATA GTTGCAGAAA CATAAATCGG
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6601   GTAAGTATTT CTGATATAGA AATGGTAAAA TTATTACTAG AACACGGTGC
       CATTCATAAA GACTATATCT TTACCATTTT AATAATGATC TTGTGCCACG
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6651   CGATATTTTA AAATGTAAAA ATCCTCCTCT TCATAAAGCT GCTAGTTTAG
       GCTATAAAAT TTTACATTTT TAGGAGGAGA AGTATTTCGA CGATCAAATC
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6701   ATAATACAGA AATTGCTAAA CTACTAATAG ATTCTGGCGC TGACATAGAA
       TATTATGTCT TTAACGATTT GATGATTATC TAAGACCGCG ACTGTATCTT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6751   CAGATACATT CTGGAAATAG TCCGTTATAT ATTTCTGTAT ATAGAAACAA
       GTCTATGTAA GACCTTTATC AGGCAATATA TAAAGACATA TATCTTTGTT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6801   TAAGTCATTA ACTAGATATT TATTAAAAAA AGGTGTTAAT TGTAATAGAT
       ATTCAGTAAT TGATCTATAA ATAATTTTTT TCCACAATTA ACATTATCTA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6851   TCTTTCTAAA TTATTACGAT GTACTGTATG ATAAGATATC TGATGATATG
       AGAAAGATTT AATAATGCTA CATGACATAC TATTCTATAG ACTACTATAC
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6901   TATAAAATAT TTATAGATTT TAATATTGAT CTTAATATAC AAACTAGAAA
       ATATTTTATA AATATCTAAA ATTATAACTA GAATTATATG TTTGATCTTT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
6951   TTTTGAAACT CCGTTACATT ACGCTATAAA GTATAAGAAT ATAGATTTAA
       AAAACTTTGA GGCAATGTAA TGCGATATTT CATATTCTTA TATCTAAATT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                           Right Arm
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7001   TTAGGATATT GTTAGATAAT AGTATTAAAA TAGATAAAAG TTTATTTTTG
       AATCCTATAA CAATCTATTA TCATAATTTT ATCTATTTTC AAATAAAAAC
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7051   CATAAACAGT ATCTCATAAA GGCACTTAAA AATAATTGTA GTTACGATAT
       GTATTTGTCA TAGAGTATTT CCGTGAATTT TTATTAACAT CAATGCTATA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7101   AATAGCGTTA CTTATAAATC ACGGAGTGCC TATAAACGAA CAAGATGATT
       TTATCGCAAT GAATATTTAG TGCCTCACGG ATATTTGCTT GTTCTACTAA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7151   TAGGTAAAAC CCCATTACAT CATTCGGTAA TTAATAGAAG AAAAGATGTA
       ATCCATTTTG GGGTAATGTA GTAAGCCATT AATTATCTTC TTTTCTACAT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7201   ACAGCACTTC TGTTAAATCT AGGAGCTGAT ATAAACGTAA TAGATGACTG
       TGTCGTGAAG ACAATTTAGA TCCTCGACTA TATTTGCATT ATCTACTGAC
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7251   TATGGGCAGT CCCTTACATT ACGCTGTTTC ACGTAACGAT ATCGAAACAA
       ATACCCGTCA GGGAATGTAA TGCGACAAAG TGCATTGCTA TAGCTTTGTT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7301   CAAAGACACT TTTAGAAAGA GGATCTAATG TTAATGTGGT TAATAATCAT
       GTTTCTGTGA AAATCTTTCT CCTAGATTAC AATTACACCA ATTATTAGTA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7351   ATAGATACCG TTCTAAATAT AGCTGTTGCA TCTAAAAACA AAACTATAGT
       TATCTATGGC AAGATTTATA TCGACAACGT AGATTTTTGT TTTGATATCA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7401   AAACTTATTA CTGAAGTACG GTACTGATAC AAAGTTGGTA GGATTAGATA
       TTTGAATAAT GACTTCATGC CATGACTATG TTTCAACCAT CCTAATCTAT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7451   AACATGTTAT TCACATAGCT ATAGAAATGA AAGATATTAA TATACTGAAT
       TTGTACAATA AGTGTATCGA TATCTTTACT TTCTATAATT ATATGACTTA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7501   GCGATCTTAT TATATGGTTG CTATGTAAAC GTCTATAATC ATAAAGGTTT
       CGCTAGAATA ATATACCAAC GATACATTTG CAGATATTAG TATTTCCAAA
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7551   CACTCCTCTA TACATGGCAG TTAGTTCTAT GAAAACAGAA TTTGTTAAAC
       GTGAGGAGAT ATGTACCGTC AATCAAGATA CTTTTGTCTT AAACAATTTG
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7601   TCTTACTTGA CCACGGTGCT TACGTAAATG CTAAAGCTAA GTTATCTGGA
       AGAATGAACT GGTGCCACGA ATGCATTTAC GATTTCGATT CAATAGACCT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
                            Right Arm
7651   AATACTCCTT TACATAAAGC TATGTTATCT AATAGTTTTA ATAATATAAA
       TTATGAGGAA ATGTATTTCG ATACAATAGA TTATCAAAAT TATTATATTT
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
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                            Right Arm
7701  ATTACTTTTA TCTTATAACG CCGACTATAA TTCTCTAAAT AATCACGGTA
      TAATGAAAAT AGAATATTGC GGCTGATATT AAGAGATTTA TTAGTGCCAT
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
7751  ATACGCCTCT AACTTGTGTT AGCTTTTTAG ATGACAAGAT AGCTATTATG
      TATGCGGAGA TTGAACACAA TCGAAAAATC TACTGTTCTA TCGATAATAC
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
7801  ATAATATCTA AAATGATGTT AGAAATATCT AAAAATCCTG AAATAGCTAA
      TATTATAGAT TTTACTACAA TCTTTATAGA TTTTTAGGAC TTTATCGATT
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
7851  TTCAGAAGGT TTTATAGTAA ACATGGAACA TATAAACAGT AATAAAAGAC
      AAGTCTTCCA AAATATCATT TGTACCTTGT ATATTTGTCA TTATTTTCTG
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
7901  TACTATCTAT AAAAGAATCA TGCGAAAAAG AACTAGATGT TATAACACAT
      ATGATAGATA TTTTCTTAGT ACGCTTTTTC TTGATCTACA ATATTGTGTA
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
7951  ATAAAGTTAA ATTCTATATA TTCTTTTAAT ATCTTTCTTG ACAATAACAT
      TATTTCAATT TAAGATATAT AAGAAAATTA TAGAAAGAAC TGTTATTGTA
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
8001  AGATCTTATG GTAAAGTTCG TAACTAATCC TAGAGTTAAT AAGATACCTG
      TCTAGAATAC CATTTCAAGC ATTGATTAGG ATCTCAATTA TTCTATGGAC
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
8051  CATGTATACG TATATATAGG GAATTAATAC GGAAAAATAA ATCATTAGCT
      GTACATATGC ATATATATCC CTTAATTATG CCTTTTTATT TAGTAATCGA
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
8101  TTTCATAGAC ATCAGCTAAT AGTTAAAGCT GTAAAAGAGA GTAAGAATCT
      AAAGTATCTG TAGTCGATTA TCAATTTCGA CATTTTCTCT CATTCTTAGA
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
8151  AGGAATAATA GGTAGGTTAC CTATAGATAT CAAACATATA ATAATGGAAC
      TCCTTATTAT CCATCCAATG GATATCTATA GTTTGTATAT TATTACCTTG
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
8201  TATTAAGTAA TAATGATTTA CATTCTGTTA TCACCAGCTG TTGTAACCCA
      ATAATTCATT ATTACTAAAT GTAAGACAAT AGTGGTCGAC AACATTGGGT
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

                            Right Arm
8251  GTAGTATAAA GAGCTCCAGC TTTTGTTCCC TTTAGTGAGG GTTAATTCCG
      CATCATATTT CTCGAGGTCG AAAACAAGGG AAATCACTCC CAATTAAGGC
      ~~~~~~~~~~~~~~~~~

         Right Arm
8301  AGCTTGGCGT AATCATGGTC ATAGCTGTTT CCTGTGTGAA ATTGTTATCC
      TCGAACCGCA TTAGTACCAG TATCGACAAA GGACACACTT TAACAATAGG
8351  GCTCACAATT CCACACAACA TACGAGCCGG AAGCATAAAG TGTAAAGCCT
      CGAGTGTTAA GGTGTGTTGT ATGCTCGGCC TTCGTATTTC ACATTTCGGA
8401  GGGGTGCCTA ATGAGTGAGC TAACTCACAT TAATTGCGTT GCGCTCACTG
      CCCCACGGAT TACTCACTCG ATTGAGTGTA ATTAACGCAA CGCGAGTGAC
8451  CCCGCTTTCC AGTCGGGAAA CCTGTCGTGC CAGCTGCATT AATGAATCGG
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GGGCGAAAGG TCAGCCCTTT GGACAGCACG GTCGACGTAA TTACTTAGCC
8501 CCAACGCGCG GGGAGAGGCG GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT
GGTTGCGCGC CCCTCTCCGC CAAACGCATA ACCCGCGAGA AGGCGAAGGA
8551 CGCTCACTGA CTCGCTGCGC TCGGTCGTTC GGCTGCGGCG AGCGGTATCA
GCGAGTGACT GAGCGACGCG AGCCAGCAAG CCGACGCCGC TCGCCATAGT
8601 GCTCACTCAA AGGCGGTAAT ACGGTTATCC ACAGAATCAG GGGATAACGC
CGAGTGAGTT TCCGCCATTA TGCCAATAGG TGTCTTAGTC CCCTATTGCG
8651 AGGAAAGAAC ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG AACCGTAAAA
TCCTTTCTTG TACACTCGTT TTCCGGTCGT TTTCCGGTCC TTGGCATTTT
8701 AGGCCGCGTT GCTGGCGTTT TTCCATAGGC TCCGCCCCCC TGACGAGCAT
TCCGGCGCAA CGACCGCAAA AAGGTATCCG AGGCGGGGGG ACTGCTCGTA
8751 CACAAAAATC GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA
GTGTTTTTAG CTGCGAGTTC AGTCTCCACC GCTTTGGGCT GTCCTGATAT
8801 AAGATACCAG GCGTTTCCCC CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC
TTCTATGGTC CGCAAAGGGG GACCTTCGAG GGAGCACGCG AGAGGACAAG
8851 CGACCCTGCC GCTTACCGGA TACCTGTCCG CCTTTCTCCC TTCGGGAAGC
GCTGGGACGG CGAATGGCCT ATGGACAGGC GGAAAGAGGG AAGCCCTTCG
8901 GTGGCGCTTT CTCATAGCTC ACGCTGTAGG TATCTCAGTT CGGTGTAGGT
CACCGCGAAA GAGTATCGAG TGCGACATCC ATAGAGTCAA GCCACATCCA
8951 CGTTCGCTCC AAGCTGGGCT GTGTGCACGA ACCCCCCGTT CAGCCCGACC
GCAAGCGAGG TTCGACCCGA CACACGTGCT TGGGGGGCAA GTCGGGCTGG
9001 GCTGCGCCTT ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC
CGACGCGGAA TAGGCCATTG ATAGCAGAAC TCAGGTTGGG CCATTCTGTG
9051 GACTTATCGC CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG
CTGAATAGCG GTGACCGTCG TCGGTGACCA TTGTCCTAAT CGTCTCGCTC
9101 GTATGTAGGC GGTGCTACAG AGTTCTTGAA GTGGTGGCCT AACTACGGCT
CATACATCCG CCACGATGTC TCAAGAACTT CACCACCGGA TTGATGCCGA
9151 ACACTAGAAG GACAGTATTT GGTATCTGCG CTCTGCTGAA GCCAGTTACC
TGTGATCTTC CTGTCATAAA CCATAGACGC GAGACGACTT CGGTCAATGG
9201 TTCGGAAAAA GAGTTGGTAG CTCTTGATCC GGCAAACAAA CCACCGCTGG
AAGCCTTTTT CTCAACCATC GAGAACTAGG CCGTTTGTTT GGTGGCGACC
9251 TAGCGGTGGT TTTTTTGTTT GCAAGCAGCA GATTACGCGC AGAAAAAAAG
ATCGCCACCA AAAAAACAAA CGTTCGTCGT CTAATGCGCG TCTTTTTTTC
9301 GATCTCAAGA AGATCCTTTG ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG
CTAGAGTTCT TCTAGGAAAC TAGAAAAGAT GCCCCAGACT GCGAGTCACC
9351 AACGAAAACT CACGTTAAGG GATTTTGGTC ATGAGATTAT CAAAAAGGAT
TTGCTTTTGA GTGCAATTCC CTAAAACCAG TACTCTAATA GTTTTTCCTA
9401 CTTCACCTAG ATCCTTTTAA ATTAAAAATG AAGTTTTAAA TCAATCTAAA
GAAGTGGATC TAGGAAAATT TAATTTTTAC TTCAAAATTT AGTTAGATTT
9451 GTATATATGA GTAAACTTGG TCTGACAGTT ACCAATGCTT AATCAGTGAG
CATATATACT CATTTGAACC AGACTGTCAA TGGTTACGAA TTAGTCACTC
9501 GCACCTATCT CAGCGATCTG TCTATTTCGT TCATCCATAG TTGCCTGACT
CGTGGATAGA GTCGCTAGAC AGATAAAGCA AGTAGGTATC AACGGACTGA
9551 CCCCGTCGTG TAGATAACTA CGATACGGGA GGGCTTACCA TCTGGCCCCA
GGGGCAGCAC ATCTATTGAT GCTATGCCCT CCCGAATGGT AGACCGGGGT
9601 GTGCTGCAAT GATACCGCGA GACCCACGCT CACCGGCTCC AGATTTATCA
CACGACGTTA CTATGGCGCT CTGGGTGCGA GTGGCCGAGG TCTAAATAGT
9651 GCAATAAACC AGCCAGCCGG AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC
CGTTATTTGG TCGGTCGGCC TTCCCGGCTC GCGTCTTCAC CAGGACGTTG
9701 TTTATCCGCC TCCATCCAGT CTATTAATTG TTGCCGGGAA GCTAGAGTAA
AAATAGGCGG AGGTAGGTCA GATAATTAAC AACGGCCCTT CGATCTCATT
9751 GTAGTTCGCC AGTTAATAGT TTGCGCAACG TTGTTGCCAT TGCTACAGGC
CATCAAGCGG TCAATTATCA AACGCGTTGC AACAACGGTA ACGATGTCCG
9801 ATCGTGGTGT CACGCTCGTC GTTTGGTATG GCTTCATTCA GCTCCGGTTC
TAGCACCACA GTGCGAGCAG CAAACCATAC CGAAGTAAGT CGAGGCCAAG

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9851  CCAACGATCA AGGCGAGTTA CATGATCCCC CATGTTGTGC AAAAAAGCGG
      GGTTGCTAGT TCCGCTCAAT GTACTAGGGG GTACAACACG TTTTTTCGCC
9901  TTAGCTCCTT CGGTCCTCCG ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG
      AATCGAGGAA GCCAGGAGGC TAGCAACAGT CTTCATTCAA CCGGCGTCAC
9951  TTATCACTCA TGGTTATGGC AGCACTGCAT AATTCTCTTA CTGTCATGCC
      AATAGTGAGT ACCAATACCG TCGTGACGTA TTAAGAGAAT GACAGTACGG
10001 ATCCGTAAGA TGCTTTTCTG TGACTGGTGA GTACTCAACC AAGTCATTCT
      TAGGCATTCT ACGAAAAGAC ACTGACCACT CATGAGTTGG TTCAGTAAGA
10051 GAGAATAGTG TATGCGGCGA CCGAGTTGCT CTTGCCCGGC GTCAATACGG
      CTCTTATCAC ATACGCCGCT GGCTCAACGA GAACGGGCCG CAGTTATGCC
10101 GATAATACCG CGCCACATAG CAGAACTTTA AAAGTGCTCA TCATTGGAAA
      CTATTATGGC GCGGTGTATC GTCTTGAAAT TTTCACGAGT AGTAACCTTT
10151 ACGTTCTTCG GGGCGAAAAC TCTCAAGGAT CTTACCGCTG TTGAGATCCA
      TGCAAGAAGC CCCGCTTTTG AGAGTTCCTA GAATGGCGAC AACTCTAGGT
10201 GTTCGATGTA ACCCACTCGT GCACCCAACT GATCTTCAGC ATCTTTTACT
      CAAGCTACAT TGGGTGAGCA CGTGGGTTGA CTAGAAGTCG TAGAAAATGA
10251 TTCACCAGCG TTTCTGGGTG AGCAAAAACA GGAAGGCAAA ATGCCGCAAA
      AAGTGGTCGC AAAGACCCAC TCGTTTTTGT CCTTCCGTTT TACGGCGTTT
10301 AAAGGGAATA AGGGCGACAC GGAAATGTTG AATACTCATA CTCTTCCTTT
      TTTCCCTTAT TCCCGCTGTG CCTTTACAAC TTATGAGTAT GAGAAGGAAA
10351 TTCAATATTA TTGAAGCATT TATCAGGGTT ATTGTCTCAT GAGCGGATAC
      AAGTTATAAT AACTTCGTAA ATAGTCCCAA TAACAGAGTA CTCGCCTATG
10401 ATATTTGAAT GTATTTAGAA AAATAAACAA ATAGGGGTTC CGCGCACATT
      TATAAACTTA CATAAATCTT TTTATTTGTT TATCCCCAAG GCGCGTGTAA
10451 TCCCCGAAAA GTGCCACCTG AGGGGCTTTT CACGGTGGAC
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FIGURE 3: Donor plasmid p1132

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                         C5 Right Arm
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     1    TGAATGTTAA ATGTTATACT TTGGATGAAG CTATAAATAT GCATTGGAAA
          ACTTACAATT TACAATATGA AACCTACTTC GATATTTATA CGTAACCTTT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    51    AATAATCCAT TTAAAGAAAG GATTCAAATA CTACAAAACC TAAGCGATAA
          TTATTAGGTA AATTTCTTTC CTAAGTTTAT GATGTTTTGG ATTCGCTATT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   101    TATGTTAACT AAGCTTATTC TTAACGACGC TTTAAATATA CACAAATAAA
          ATACAATTGA TTCGAATAAG AATTGCTGCG AAATTTATAT GTGTTTATTT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   151    CATAATTTTT GTATAACCTA ACAAATAACT AAAACATAAA AATAATAAAA
          GTATTAAAAA CATATTGGAT TGTTTATTGA TTTTGTATTT TTATTATTTT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   201    GGAAATGTAA TATCGTAATT ATTTTACTCA GGAATGGGGT TAAATATTTA
          CCTTTACATT ATAGCATTAA TAAAATGAGT CCTTACCCCA ATTTATAAAT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   251    TATCACGTGT ATATCTATAC TGTTATCGTA TACTCTTTAC AATTACTATT
          ATAGTGCACA TATAGATATG ACAATAGCAT ATGAGAAATG TTAATGATAA
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   301    ACGAATATGC AAGAGATAAT AAGATTACGT ATTTAAGAGA ATCTTGTCAT
          TGCTTATACG TTCTCTATTA TTCTAATGCA TAAATTCTCT TAGAACAGTA
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   351    GATAATTGGG TACGACATAG TGATAAATGC TATTTCGCAT CGTTACATAA
          CTATTAACCC ATGCTGTATC ACTATTTACG ATAAAGCGTA GCAATGTATT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   401    AGTCAGTTGG AAAGATGGAT TTGACAGATG TAACTTAATA GGTGCAAAAA
          TCAGTCAACC TTTCTACCTA AACTGTCTAC ATTGAATTAT CCACGTTTTT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   451    TGTTAAATAA CAGCATTCTA TCGGAAGATA GGATACCAGT TATATTATAC
          ACAATTTATT GTCGTAAGAT AGCCTTCTAT CCTATGGTCA ATATAATATG
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   501    AAAAATCACT GGTTGGATAA AACAGATTCT GCAATATTCG TAAAAGATGA
          TTTTTAGTGA CCAACCTATT TTGTCTAAGA CGTTATAAGC ATTTTCTACT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   551    AGATTACTGC GAATTTGTAA ACTATGACAA TAAAAAGCCA TTTATCTCAA
          TCTAATGACG CTTAAACATT TGATACTGTT ATTTTTCGGT AAATAGAGTT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   601    CGACATCGTG TAATTCTTCC ATGTTTTATG TATGTGTTTC AGATATTATG
          GCTGTAGCAC ATTAAGAAGG TACAAAATAC ATACACAAAG TCTATAATAC
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                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 651 AGATTACTAT AAACTTTTTG TATACTTATA TTCCGTAAAC TATATTAATC
     TCTAATGATA TTTGAAAAAC ATATGAATAT AAGGCATTTG ATATAATTAG
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 701 ATGAAGAAAA TGAAAAAGTA TAGAAGCTGT TCACGAGCGG TTGTTGAAAA
     TACTTCTTTT ACTTTTTCAT ATCTTCGACA AGTGCTCGCC AACAACTTTT
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 751 CAACAAAATT ATACATTCAA GATGGCTTAC ATATACGTCT GTGAGGCTAT
     GTTGTTTTAA TATGTAAGTT CTACCGAATG TATATGCAGA CACTCCGATA
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 801 CATGGATAAT GACAATGCAT CTCTAAATAG GTTTTTGGAC AATGGATTCG
     GTACCTATTA CTGTTACGTA GAGATTTATC CAAAAACCTG TTACCTAAGC
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 851 ACCCTAACAC GGAATATGGT ACTCTACAAT CTCCTCTTGA AATGGCTGTA
     TGGGATTGTG CCTTATACCA TGAGATGTTA GAGGAGAACT TTACCGACAT
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 901 ATGTTCAAGA ATACCGAGGC TATAAAAATC TTGATGAGGT ATGGAGCTAA
     TACAAGTTCT TATGGCTCCG ATATTTTTAG AACTACTCCA TACCTCGATT
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 951 ACCTGTAGTT ACTGAATGCA CAACTTCTTG TCTGCATGAT GCGGTGTTGA
     TGGACATCAA TGACTTACGT GTTGAAGAAC AGACGTACTA CGCCACAACT
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1001 GAGACGACTA CAAAATAGTG AAAGATCTGT TGAAGAATAA CTATGTAAAC
     CTCTGCTGAT GTTTTATCAC TTTCTAGACA ACTTCTTATT GATACATTTG
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1051 AATGTTCTTT ACAGCGGAGG CTTTACTCCT TTGTGTTTGG CAGCTTACCT
     TTACAAGAAA TGTCGCCTCC GAAATGAGGA AACACAAACC GTCGAATGGA
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1101 TAACAAAGTT AATTTGGTTA AACTTCTATT GGCTCATTCG GCGGATGTAG
     ATTGTTTCAA TTAAACCAAT TTGAAGATAA CCGAGTAAGC CGCCTACATC
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1151 ATATTTCAAA CACGGATCGG TTAACTCCTC TACATATAGC CGTATCAAAT
     TATAAAGTTT GTGCCTAGCC AATTGAGGAG ATGTATATCG GCATAGTTTA
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1201 AAAAATTTAA CAATGGTTAA ACTTCTATTG AACAAAGGTG CTGATACTGA
     TTTTTAAATT GTTACCAATT TGAAGATAAC TTGTTTCCAC GACTATGACT
                         C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1251 CTTGCTGGAT AACATGGGAT GTACTCCTTT AATGATCGCT GTACAATCTG
     GAACGACCTA TTGTACCCTA CATGAGGAAA TTACTAGCGA CATGTTAGAC
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                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1301 GAAATATTGA AATATGTAGC ACACTACTTA AAAAAAATAA AATGTCCAGA
        CTTTATAACT TTATACATCG TGTGATGAAT TTTTTTTATT TTACAGGTCT
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1351 ACTGGGAAAA ATTGATCTTG CCAGCTGTAA TTCATGGTAG AAAAGAAGTG
        TGACCCTTTT TAACTAGAAC GGTCGACATT AAGTACCATC TTTTCTTCAC
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1401 CTCAGGCTAC TTTTCAACAA AGGAGCAGAT GTAAACTACA TCTTTGAAAG
        GAGTCCGATG AAAAGTTGTT TCCTCGTCTA CATTTGATGT AGAAACTTTC
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1451 AAATGGAAAA TCATATACTG TTTTGGAATT GATTAAAGAA AGTTACTCTG
        TTTACCTTTT AGTATATGAC AAAACCTTAA CTAATTTCTT TCAATGAGAC
                C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1501 AGACACAAAA GAGGTAGCTG AAGTGGTACT CTCAAAGGTA CGTGACTAAT
        TCTGTGTTTT CTCCATCGAC TTCACCATGA GAGTTTCCAT GCACTGATTA
                                                Repeat Region
                                                ~~~~~~~~~~
   1551 TAGCTATAAA AAGGATCGGC CGCTCTAGAA CTAGTGGATC GGGTTCTTTA
        ATCGATATTT TTCCTAGCCG GCGAGATCTT GATCACCTAG CCCAAGAAAT
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1601 TTCTATACTT AAAAAGTGAA AATAAATACA AAGGTTCTTG AGGGTTGTGT
        AAGATATGAA TTTTTCACTT TTATTTATGT TTCCAAGAAC TCCCAACACA
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1651 TAAATTGAAA GCGAGAAATA ATCATAAATT ATTTCATTAT CGCGATATCC
        ATTTAACTTT CGCTCTTTAT TAGTATTTAA TAAAGTAATA GCGCTATAGG
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1701 GTTAAGTTTG TATCGTACCC CGATCCCCCG AGCCATGCAG GCCGAAGGCC
        CAATTCAAAC ATAGCATGGG GCTAGGGGGC TCGGTACGTC CGGCTTCCGG
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1751 GGGGCACAGG GGGTTCGACG GGCGATGCTG ATGGCCCAGG AGGCCCTGGC
        CCCCGTGTCC CCCAAGCTGC CCGCTACGAC TACCGGGTCC TCCGGGACCG
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1801 ATTCCTGATG GCCCAGGGGG CAATGCTGGC GGCCCAGGAG AGGCGGGTGC
        TAAGGACTAC CGGGTCCCCC GTTACGACCG CCGGGTCCTC TCCGCCCACG
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1851 CACGGGCGGC AGAGGTCCCC GGGGCGCAGG GGCAGCAAGG GCCTCGGGGC
        GTGCCCGCCG TCTCCAGGGG CCCCGCGTCC CCGTCGTTCC CGGAGCCCCG
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1901 CGGGAGGAGG CGCCCCGCGG GGTCCGCATG GCGGCGCGGC TTCAGGGCTG
        GCCCTCCTCC GCGGGGCGCC CCAGGCGTAC CGCCGCGCCG AAGTCCCGAC
                        Repeat Region
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1951 AATGGATGCT GCAGATGCGG GGCCAGGGGG CCGGAGAGCC GCCTGCTTGA
        TTACCTACGA CGTCTACGCC CCGGTCCCCC GGCCTCTCGG CGGACGAACT
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                Repeat Region
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2001   GTTCTACCTC GCCATGCCTT TCGCGACACC CATAGCTTGA TATCGAATTC
           CAAGATGGAG CGGTACGGAA AGCGCTGTGG GTATCGAACT ATAGCTTAAG
                                            C1B promoter
                                  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2051   TAGGGGGATC CACTAGTTCT AGAGGATCAT TATTTAACGT AAACTAAATG
           ATCCCCCTAG GTGATCAAGA TCTCCTAGTA ATAAATTGCA TTTGATTTAC
                               C1B promoter
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2101   GAAAAGCTAT TTACAGGTAC ATACGGTGTT TTTCTGGAAT CAAATGATTC
           CTTTTCGATA AATGTCCATG TATGCCACAA AAAGACCTTA GTTTACTAAG
                               C1B promoter
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2151   TGATTTTGAG GATTTTATCA ATACAATAAT GACAGTGCTA ACTGGTAAAA
           ACTAAAACTC CTAAAATAGT TATGTTATTA CTGTCACGAT TGACCATTTT
                               C1B promoter
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2201   AAGAAAGCAA ACAATTATCA TGGCTAACAA TTTTTATTAT ATTTGTAGTA
           TTCTTTCGTT TGTTAATAGT ACCGATTGTT AAAAATAATA TAAACATCAT
                               C1B promoter
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2251   TGCATAGTGG TCTTTACGTT TCTTTATTTA AAGTTAATGT GTTAAGATTA
           ACGTATCACC AGAAATGCAA AGAAATAAAT TTCAATTACA CAATTCTAAT
            C1B promoter                              LacZ
           ~~~~~~~~~~~~~~~~~~         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2301   AATGGAGTAA TTGGATCCCC CATCGATGGG GAATTCACTG GCCGTCGTTT
           TTACCTCATT AACCTAGGGG GTAGCTACCC CTTAAGTGAC CGGCAGCAAA
                                   LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2351   TACAACGTCG TGACTGGGAA AACCCTGGCG TTACCCAACT TAATCGCCTT
           ATGTTGCAGC ACTGACCCTT TTGGGACCGC AATGGGTTGA ATTAGCGGAA
                                   LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2401   GCAGCACATC CCCCTTTCGC CAGCTGGCGT AATAGCGAAG AGGCCCGCAC
           CGTCGTGTAG GGGGAAAGCG GTCGACCGCA TTATCGCTTC TCCGGGCGTG
                                   LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2451   CGATCGCCCT TCCCAACAGT TGCGCAGCCT GAATGGCGAA TGGCGCTTTG
           GCTAGCGGGA AGGGTTGTCA ACGCGTCGGA CTTACCGCTT ACCGCGAAAC
                                   LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2501   CCTGGTTTCC GGCACCAGAA GCGGTGCCGG AAAGCTGGCT GGAGTGCGAT
           GGACCAAAGG CCGTGGTCTT CGCCACGGCC TTTCGACCGA CCTCACGCTA
                                   LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2551   CTTCCTGAGG CCGATACTGT CGTCGTCCCC TCAAACTGGC AGATGCACGG
           GAAGGACTCC GGCTATGACA GCAGCAGGGG AGTTTGACCG TCTACGTGCC
                                   LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2601   TTACGATGCG CCCATCTACA CCAACGTGAC CTATCCCATT ACGGTCAATC
           AATGCTACGC GGGTAGATGT GGTTGCACTG GATAGGGTAA TGCCAGTTAG
                                   LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    2651   CGCCGTTTGT TCCCACGGAG AATCCGACGG GTTGTTACTC GCTCACATTT
           GCGGCAAACA AGGGTGCCTC TTAGGCTGCC CAACAATGAG CGAGTGTAAA
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                              LacZ
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2701    AATGTTGATG AAAGCTGGCT ACAGGAAGGC CAGACGCGAA TTATTTTTGA
        TTACAACTAC TTTCGACCGA TGTCCTTCCG GTCTGCGCTT AATAAAAACT
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2751    TGGCGTTAAC TCGGCGTTTC ATCTGTGGTG CAACGGGCGC TGGGTCGGTT
        ACCGCAATTG AGCCGCAAAG TAGACACCAC GTTGCCCGCG ACCCAGCCAA
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2801    ACGGCCAGGA CAGTCGTTTG CCGTCTGAAT TTGACCTGAG CGCATTTTTA
        TGCCGGTCCT GTCAGCAAAC GGCAGACTTA AACTGGACTC GCGTAAAAAT
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2851    CGCGCCGGAG AAAACCGCCT CGCGGTGATG GTGCTGCGCT GGAGTGACGG
        GCGCGGCCTC TTTTGGCGGA GCGCCACTAC CACGACGCGA CCTCACTGCC
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2901    CAGTTATCTG GAAGATCAGG ATATGTGGCG GATGAGCGGC ATTTTCCGTG
        GTCAATAGAC CTTCTAGTCC TATACACCGC CTACTCGCCG TAAAAGGCAC
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2951    ACGTCTCGTT GCTGCATAAA CCGACTACAC AAATCAGCGA TTTCCATGTT
        TGCAGAGCAA CGACGTATTT GGCTGATGTG TTTAGTCGCT AAAGGTACAA
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3001    GCCACTCGCT TTAATGATGA TTTCAGCCGC GCTGTACTGG AGGCTGAAGT
        CGGTGAGCGA AATTACTACT AAAGTCGGCG CGACATGACC TCCGACTTCA
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3051    TCAGATGTGC GGCGAGTTGC GTGACTACCT ACGGGTAACA GTTTCTTTAT
        AGTCTACACG CCGCTCAACG CACTGATGGA TGCCCATTGT CAAAGAAATA
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3101    GGCAGGGTGA AACGCAGGTC GCCAGCGGCA CCGCGCCTTT CGGCGGTGAA
        CCGTCCCACT TTGCGTCCAG CGGTCGCCGT GGCGCGGAAA GCCGCCACTT
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3151    ATTATCGATG AGCGTGGTGG TTATGCCGAT CGCGTCACAC TACGTCTGAA
        TAATAGCTAC TCGCACCACC AATACGGCTA GCGCAGTGTG ATGCAGACTT
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3201    CGTCGAAAAC CCGAAACTGT GGAGCGCCGA AATCCCGAAT CTCTATCGTG
        GCAGCTTTTG GGCTTTGACA CCTCGCGGCT TTAGGGCTTA GAGATAGCAC
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3251    CGGTGGTTGA ACTGCACACC GCCGACGGCA CGCTGATTGA AGCAGAAGCC
        GCCACCAACT TGACGTGTGG CGGCTGCCGT GCGACTAACT TCGTCTTCGG
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3301    TGCGATGTCG GTTTCCGCGA GGTGCGGATT GAAAATGGTC TGCTGCTGCT
        ACGCTACAGC CAAAGGCGCT CCACGCCTAA CTTTTACCAG ACGACGACGA
                              LacZ
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
3351    GAACGGCAAG CCGTTGCTGA TTCGAGGCGT TAACCGTCAC GAGCATCATC
        CTTGCCGTTC GGCAACGACT AAGCTCCGCA ATTGGCAGTG CTCGTAGTAG
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                                   LacZ
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   3401   CTCTGCATGG TCAGGTCATG GATGAGCAGA CGATGGTGCA GGATATCCTG
          GAGACGTACC AGTCCAGTAC CTACTCGTCT GCTACCACGT CCTATAGGAC
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3451   CTGATGAAGC AGAACAACTT TAACGCCGTG CGCTGTTCGC ATTATCCGAA
          GACTACTTCG TCTTGTTGAA ATTGCGGCAC GCGACAAGCG TAATAGGCTT
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3501   CCATCCGCTG TGGTACACGC TGTGCGACCG CTACGGCCTG TATGTGGTGG
          GGTAGGCGAC ACCATGTGCG ACACGCTGGC GATGCCGGAC ATACACCACC
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3551   ATGAAGCCAA TATTGAAACC CACGGCATGG TGCCAATGAA TCGTCTGACC
          TACTTCGGTT ATAACTTTGG GTGCCGTACC ACGGTTACTT AGCAGACTGG
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3601   GATGATCCGC GCTGGCTACC GGCGATGAGC GAACGCGTAA CGCGAATGGT
          CTACTAGGCG CGACCGATGG CCGCTACTCG CTTGCGCATT GCGCTTACCA
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3651   GCAGCGCGAT CGTAATCACC CGAGTGTGAT CATCTGGTCG CTGGGGAATG
          CGTCGCGCTA GCATTAGTGG GCTCACACTA GTAGACCAGC GACCCCTTAC
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3701   AATCAGGCCA CGGCGCTAAT CACGACGCGC TGTATCGCTG GATCAAATCT
          TTAGTCCGGT GCCGCGATTA GTGCTGCGCG ACATAGCGAC CTAGTTTAGA
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3751   GTCGATCCTT CCCGCCCGGT GCAGTATGAA GGCGGCGGAG CCGACACCAC
          CAGCTAGGAA GGGCGGGCCA CGTCATACTT CCGCCGCCTC GGCTGTGGTG
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3801   GGCCACCGAT ATTATTTGCC CGATGTACGC GCGCGTGGAT GAAGACCAGC
          CCGGTGGCTA TAATAAACGG GCTACATGCG CGCGCACCTA CTTCTGGTCG
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3851   CCTTCCCGGC TGTGCCGAAA TGGTCCATCA AAAAATGGCT TTCGCTACCT
          GGAAGGGCCG ACACGGCTTT ACCAGGTAGT TTTTTACCGA AAGCGATGGA
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3901   GGAGAGACGC GCCCGCTGAT CCTTTGCGAA TACGCCCACG CGATGGGTAA
          CCTCTCTGCG CGGGCGACTA GGAAACGCTT ATGCGGGTGC GCTACCCATT
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   3951   CAGTCTTGGC GGTTTCGCTA AATACTGGCA GGCGTTTCGT CAGTATCCCC
          GTCAGAACCG CCAAAGCGAT TTATGACCGT CCGCAAAGCA GTCATAGGGG
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   4001   GTTTACAGGG CGGCTTCGTC TGGGACTGGG TGGATCAGTC GCTGATTAAA
          CAAATGTCCC GCCGAAGCAG ACCCTGACCC ACCTAGTCAG CGACTAATTT
                                   LacZ
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   4051   TATGATGAAA ACGGCAACCC GTGGTCGGCT TACGGCGGTG ATTTGGCGA
          ATACTACTTT TGCCGTTGGG CACCAGCCGA ATGCCGCCAC TAAAACCGCT
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                            LacZ
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    4101   TACGCCGAAC GATCGCCAGT TCTGTATGAA CGGTCTGGTC TTTGCCGACC
           ATGCGGCTTG CTAGCGGTCA AGACATACTT GCCAGACCAG AAACGGCTGG
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4151   GCACGCCGCA TCCAGCGCTG ACGGAAGCAA AACACCAGCA GCAGTTTTTC
           CGTGCGGCGT AGGTCGCGAC TGCCTTCGTT TTGTGGTCGT CGTCAAAAAG
                            LacZ
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    4201   CAGTTCCGTT TATCCGGGCA AACCATCGAA GTGACCAGCG AATACCTGTT
           GTCAAGGCAA ATAGGCCCGT TTGGTAGCTT CACTGGTCGC TTATGGACAA
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4251   CCGTCATAGC GATAACGAGC TCCTGCACTG GATGGTGGCG CTGGATGGTA
           GGCAGTATCG CTATTGCTCG AGGACGTGAC CTACCACCGC GACCTACCAT
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4301   AGCCGCTGGC AAGCGGTGAA GTGCCTCTGG ATGTCGCTCC ACAAGGTAAA
           TCGGCGACCG TTCGCCACTT CACGGAGACC TACAGCGAGG TGTTCCATTT
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4351   CAGTTGATTG AACTGCCTGA ACTACCGCAG CCGGAGAGCG CCGGGCAACT
           GTCAACTAAC TTGACGGACT TGATGGCGTC GGCCTCTCGC GGCCCGTTGA
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4401   CTGGCTCACA GTACGCGTAG TGCAACCGAA CGCGACCGCA TGGTCAGAAG
           GACCGAGTGT CATGCGCATC ACGTTGGCTT GCGCTGGCGT ACCAGTCTTC
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4451   CCGGGCACAT CAGCGCCTGG CAGCAGTGGC GTCTGGCGGA AAACCTCAGT
           GGCCCGTGTA GTCGCGGACC GTCGTCACCG CAGACCGCCT TTTGGAGTCA
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4501   GTGACGCTCC CCGCCGCGTC CCACGCCATC CCGCATCTGA CCACCAGCGA
           CACTGCGAGG GGCGGCGCAG GGTGCGGTAG GGCGTAGACT GGTGGTCGCT
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4551   AATGGATTTT TGCATCGAGC TGGGTAATAA GCGTTGGCAA TTTAACCGCC
           TTACCTAAAA ACGTAGCTCG ACCCATTATT CGCAACCGTT AAATTGGCGG
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4601   AGTCAGGCTT TCTTTCACAG ATGTGGATTG GCGATAAAAA ACAACTGCTG
           TCAGTCCGAA AGAAAGTGTC TACACCTAAC CGCTATTTTT TGTTGACGAC
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4651   ACGCCGCTGC GCGATCAGTT CACCCGTGCA CCGCTGGATA ACGACATTGG
           TGCGGCGACG CGCTAGTCAA GTGGGCACGT GGCGACCTAT TGCTGTAACC
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4701   CGTAAGTGAA GCGACCCGCA TTGACCCTAA CGCCTGGGTC GAACGCTGGA
           GCATTCACTT CGCTGGGCGT AACTGGGATT GCGGACCCAG CTTGCGACCT
                            LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4751   AGGCGGCGGG CCATTACCAG GCCGAAGCAG CGTTGTTGCA GTGCACGGCA
           TCCGCCGCCC GGTAATGGTC CGGCTTCGTC GCAACAACGT CACGTGCCGT
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                                 LacZ
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    4801   GATACACTTG CTGATGCGGT GCTGATTACG ACCGCTCACG CGTGGCAGCA
           CTATGTGAAC GACTACGCCA CGACTAATGC TGGCGAGTGC GCACCGTCGT
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4851   TCAGGGGAAA ACCTTATTTA TCAGCCGGAA AACCTACCGG ATTGATGGTA
           AGTCCCCTTT TGGAATAAAT AGTCGGCCTT TTGGATGGCC TAACTACCAT
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4901   GTGGTCAAAT GGCGATTACC GTTGATGTTG AAGTGGCGAG CGATACACCG
           CACCAGTTTA CCGCTAATGG CAACTACAAC TTCACCGCTC GCTATGTGGC
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4951   CATCCGGCGC GGATTGGCCT GAACTGCCAG CTGGCGCAGG TAGCAGAGCG
           GTAGGCCGCG CCTAACCGGA CTTGACGGTC GACCGCGTCC ATCGTCTCGC
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5001   GGTAAACTGG CTCGGATTAG GGCCGCAAGA AAACTATCCC GACCGCCTTA
           CCATTTGACC GAGCCTAATC CCGGCGTTCT TTTGATAGGG CTGGCGGAAT
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5051   CTGCCGCCTG TTTTGACCGC TGGGATCTGC CATTGTCAGA CATGTATACC
           GACGGCGGAC AAAACTGGCG ACCCTAGACG GTAACAGTCT GTACATATGG
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5101   CCGTACGTCT TCCCGAGCGA AAACGGTCTG CGCTGCGGGA CGCGCGAATT
           GGCATGCAGA AGGGCTCGCT TTTGCCAGAC GCGACGCCCT GCGCGCTTAA
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5151   GAATTATGGC CCACACCAGT GGCGCGGCGA CTTCCAGTTC AACATCAGCC
           CTTAATACCG GGTGTGGTCA CCGCGCCGCT GAAGGTCAAG TTGTAGTCGG
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5201   GGTACAGTCA ACAGCAATTG ATGGAAACCA GCCATTCGCC ATCTGCTGCA
           CCATGTCAGT TGTCGTTAAC TACCTTTGGT CGGTAAGCGG TAGACGACGT
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5251   CGCGGAAGAG GCACATGGCT GAATATCGAC GGTTTCCATA TGGGGATTGG
           GCGCCTTCTC CGTGTACCGA CTTATAGCTG CCAAAGGTAT ACCCCTAACC
                                 LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5301   TGGCGACGAC TCCTGGAGCC CGTCAGTATC GGCGGAATTC CAGCTGAGCG
           ACCGCTGCTG AGGACCTCGG GCAGTCATAG CCGCCTTAAG GTCGACTCGC
                           LacZ
           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    5351   CCGGTCGCTA CCATTACCAG TTGGTCTGGT GTCAAAAATA ATAATAACCG
           GGCCAGCGAT GGTAATGGTC AACCAGACCA CAGTTTTTAT TATTATTGGC
    5401   GGCAGGGGGG ATCCGGAGCT TATCGCAGAT CAATTCGATA TCAAGCTTAT
           CCGTCCCCCC TAGGCCTCGA ATAGCGTCTA GTTAAGCTAT AGTTCGAATA
                                                  H6  Promoter
                                                  ~~~~~~~~~~~~~
    5451   CGATACCGTC GACGGTATCG ATAAGCTCTA GTGGAGGGTT CTTTATTCTA
           GCTATGGCAG CTGCCATAGC TATTCGAGAT CACCTCCCAA GAAATAAGAT
                           H6  Promoter
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5501  TACTTAAAAA GTGAAAATAA ATACAAAGGT TCTTGAGGGT TGTGTTAAAT
      ATGAATTTTT CACTTTTATT TATGTTTCCA AGAACTCCCA ACACAATTTA
                          H6  Promoter
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5551  TGAAAGCGAG AAATAATCAT AAATTATTTC ATTATCGCGA TATCCGTTAA
      ACTTTCGCTC TTTATTAGTA TTTAATAAAG TAATAGCGCT ATAGGCAATT
       H6  Promoter                           NYESO-1
      ~~~~~~~~~~~~~~~~            ~~~~~~~~~~~~~~~~~~~~~~~~~~~
5601  GTTTGTATCG TACCCCCCCC GAGCCATGCA GGCCGAAGGC CGGGGCACAG
      CAAACATAGC ATGGGGGGGG CTCGGTACGT CCGGCTTCCG GCCCCGTGTC
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5651  GGGGTTCGAC GGGCGATGCT GATGGCCCAG GAGGCCCTGG CATTCCTGAT
      CCCCAAGCTG CCCGCTACGA CTACCGGGTC CTCCGGGACC GTAAGGACTA
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5701  GGCCCAGGGG GCAATGCTGG CGGCCCAGGA GAGGCGGGTG CCACGGGCGG
      CCGGGTCCCC CGTTACGACC GCCGGGTCCT CTCCGCCCAC GGTGCCCGCC
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5751  CAGAGGTCCC CGGGGCGCAG GGGCAGCAAG GGCCTCGGGG CCGGGAGGAG
      GTCTCCAGGG GCCCCGCGTC CCCGTCGTTC CCGGAGCCCC GGCCCTCCTC
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5801  GCGCCCCGCG GGGTCCGCAT GGCGGCGCGG CTTCAGGGCT GAATGGATGC
      CGCGGGGCGC CCCAGGCGTA CCGCCGCGCC GAAGTCCCGA CTTACCTACG
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5851  TGCAGATGCG GGGCCAGGGG GCCGGAGAGC CGCCTGCTTG AGTTCTACCT
      ACGTCTACGC CCCGGTCCCC CGGCCTCTCG GCGGACGAAC TCAAGATGGA
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5901  CGCCATGCCT TTCGCGACAC CCATGGAAGC AGAGCTGGCC CGCAGGAGCC
      GCGGTACGGA AAGCGCTGTG GGTACCTTCG TCTCGACCGG GCGTCCTCGG
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5951  TGGCCCAGGA TGCCCCACCG CTTCCCGTGC CAGGGGTGCT TCTGAAGGAG
      ACCGGGTCCT ACGGGGTGGC GAAGGGCACG GTCCCCACGA AGACTTCCTC
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6001  TTCACTGTGT CCGGCAACAT ACTGACTATC CGACTGACTG CTGCAGACCA
      AAGTGACACA GGCCGTTGTA TGACTGATAG GCTGACTGAC GACGTCTGGT
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6051  CCGCCAACTG CAGCTCTCCA TCAGCTCCTG TCTCCAGCAG CTTTCCCTGT
      GGCGGTTGAC GTCGAGAGGT AGTCGAGGAC AGAGGTCGTC GAAAGGGACA
                             NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6101  TGATGTGGAT CACGCAGGTG TTTCTGCCCG TGTTTTTGGC TCAGCCTCCC
      ACTACACCTA GTGCGTCCAC AAAGACGGGC ACAAAAACCG AGTCGGAGGG
                 NYESO-1
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~
6151  TCAGGGCAGA GGCGCTAAGT AATTAATTTT TTTTTGGGCT GCAGGATCGC
      AGTCCCGTCT CCGCGATTCA TTAATTAAAA AAAAACCCGA CGTCCTAGCG
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                         sE/L Promoter
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6201  TAGCAAAAAT TGAAATTTTA TTTTTTTTTT TTGGAATATA AATAAGCTCG
      ATCGTTTTTA ACTTTAAAAT AAAAAAAAAA AACCTTATAT TTATTCGAGC
                                  hTRP-2
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      sE/L Promoter
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6251  AAGCTCGAGC CATGAGCCCC CTTTGGTGGG GGTTTCTGCT CAGTTGCTTG
      TTCGAGCTCG GTACTCGGGG GAAACCACCC CCAAAGACGA GTCAACGAAC
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6301  GGCTGCAAAA TCCTGCCAGG AGCCCAGGGT CAGTTCCCCC GAGTCTGCAT
      CCGACGTTTT AGGACGGTCC TCGGGTCCCA GTCAAGGGGG CTCAGACGTA
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6351  GACGGTGGAC AGCCTAGTGA ACAAGGAGTG CTGCCCACGC CTGGGTGCAG
      CTGCCACCTG TCGGATCACT TGTTCCTCAC GACGGGTGCG GACCCACGTC
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6401  AGTCGGCCAA TGTCTGTGGC TCTCAGCAAG GCCGGGGGCA GTGCACAGAG
      TCAGCCGGTT ACAGACACCG AGAGTCGTTC CGGCCCCCGT CACGTGTCTC
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6451  GTGCGAGCCG ACACAAGGCC CTGGAGTGGT CCCTACATCC TACGAAACCA
      CACGCTCGGC TGTGTTCCGG GACCTCACCA GGGATGTAGG ATGCTTTGGT
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6501  GGATGACCGT GAGCTGTGGC CAAGAAAATT CTTCCACCGG ACCTGCAAGT
      CCTACTGGCA CTCGACACCG GTTCTTTTAA GAAGGTGGCC TGGACGTTCA
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6551  GCACAGGAAA CTTTGCCGGC TATAATTGTG GAGACTGCAA GTTTGGCTGG
      CGTGTCCTTT GAAACGGCCG ATATTAACAC CTCTGACGTT CAAACCGACC
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6601  ACCGGTCCCA ACTGCGAGCG GAAGAAACCA CCAGTGATTC GGCAGAACAT
      TGGCCAGGGT TGACGCTCGC CTTCTTTGGT GGTCACTAAG CCGTCTTGTA
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6651  CCATTCCTTG AGTCCTCAGG AAAGAGAGCA GTTCTTGGGC GCCTTAGATC
      GGTAAGGAAC TCAGGAGTCC TTTCTCTCGT CAAGAACCCG CGGAATCTAG
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6701  TCGCGAAGAA GAGAGTACAC CCCGACTACG TGATCACCAC ACAACACTGG
      AGCGCTTCTT CTCTCATGTG GGGCTGATGC ACTAGTGGTG TGTTGTGACC
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6751  CTGGGCCTGC TTGGGCCCAA TGGAACCCAG CCGCAGTTTG CCAACTGCAG
      GACCCGGACG AACCCGGGTT ACCTTGGGTC GGCGTCAAAC GGTTGACGTC
                            hTRP-2
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6801  TGTTTATGAT TTCTTCGTGT GGCTCCATTA TTATTCTGTT AGAGATACAT
      ACAAATACTA AAGAAGCACA CCGAGGTAAT AATAAGACAA TCTCTATGTA
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                          hTRP-2
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6851   TATTAGGACC AGGACGCCCC TACAGGGCCA TAGATTTCTC ACATCAAGGA
       ATAATCCTGG TCCTGCGGGG ATGTCCCGGT ATCTAAAGAG TGTAGTTCCT
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6901   CCTGCATTTG TTACCTGGCA CCGGTACCAT TTGTTGTGTC TGGAAAGAGA
       GGACGTAAAC AATGGACCGT GGCCATGGTA AACAACACAG ACCTTTCTCT
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6951   TCTCCAGCGA CTCATTGGCA ATGAGTCTTT TGCTTTGCCC TACTGGAACT
       AGAGGTCGCT GAGTAACCGT TACTCAGAAA ACGAAACGGG ATGACCTTGA
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7001   TTGCCACTGG GAGGAACGAG TGTGATGTGT GTACAGACCA GCTGTTTGGG
       AACGGTGACC CTCCTTGCTC ACACTACACA CATGTCTGGT CGACAAACCC
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7051   GCAGCGAGAC CAGACGATCC GACTCTGATT AGTCGGAACT CAAGATTCTC
       CGTCGCTCTG GTCTGCTAGG CTGAGACTAA TCAGCCTTGA GTTCTAAGAG
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7101   CAGCTGGGAA ACTGTCTGTG ATAGCTTGGA TGACTACAAC CACCTGGTCA
       GTCGACCCTT TGACAGACAC TATCGAACCT ACTGATGTTG GTGGACCAGT
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7151   CCTTGTGCAA TGGAACCTAT GAAGGTTTGC TGAGAAGAAA TCAAATGGGA
       GGAACACGTT ACCTTGGATA CTTCCAAACG ACTCTTCTTT AGTTTACCCT
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7201   AGAAACAGCA TGAAATTGCC AACCTTAAAA GACATACGAG ATTGCCTGTC
       TCTTTGTCGT ACTTTAACGG TTGGAATTTT CTGTATGCTC TAACGGACAG
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7251   TCTCCAGAAG TTTGACAATC CTCCCTTCTT CCAGAACTCT ACCTTCAGTT
       AGAGGTCTTC AAACTGTTAG GAGGGAAGAA GGTCTTGAGA TGGAAGTCAA
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7301   TCAGGAATGC TTTGGAAGGG TTTGATAAAG CAGATGGGAC TCTGGATTCT
       AGTCCTTACG AAACCTTCCC AAACTATTTC GTCTACCCTG AGACCTAAGA
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7351   CAAGTGATGA GCCTTCATAA TTTGGTTCAT TCCTTCCTGA ACGGGACAAA
       GTTCACTACT CGGAAGTATT AAACCAAGTA AGGAAGGACT TGCCCTGTTT
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7401   CGCTTTGCCA CATTCAGCCG CCAATGATCC CATCTTCGTG GTGATTTCTA
       GCGAAACGGT GTAAGTCGGC GGTTACTAGG GTAGAAGCAC CACTAAAGAT
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7451   ATCGTTTGCT TTACAATGCT ACAACAAACA TCCTTGAACA TGTAAGAAAA
       TAGCAAACGA AATGTTACGA TGTTGTTTGT AGGAACTTGT ACATTCTTTT
                          hTRP-2
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
7501   GAGAAAGCGA CCAAGGAACT CCCTTCCCTG CATGTGCTGG TTCTTCATTC
       CTCTTTCGCT GGTTCCTTGA GGGAAGGGAC GTACACGACC AAGAAGTAAG
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                                    hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7551       CTTTACTGAT GCCATCTTTG ATGAGTGGAT GAAAAGATTT AATCCTCCTG
               GAAATGACTA CGGTAGAAAC TACTCACCTA CTTTTCTAAA TTAGGAGGAC
                                    hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7601       CAGATGCCTG GCCTCAGGAG CTGGCCCCTA TTGGTCACAA TCGGATGTAC
               GTCTACGGAC CGGAGTCCTC GACCGGGGAT AACCAGTGTT AGCCTACATG
                                    hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7651       AACATGGTTC CTTTCTTCCC TCCAGTGACT AATGAAGAAC TCTTTTTAAC
               TTGTACCAAG GAAAGAAGGG AGGTCACTGA TTACTTCTTG AGAAAAATTG
                                    hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7701       CTCAGACCAA CTTGGCTACA GCTATGCCAT CGATCTGCCA GTTTCAGTTG
               GAGTCTGGTT GAACCGATGT CGATACGGTA GCTAGACGGT CAAAGTCAAC
                                    hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7751       AAGAAACTCC AGGTTGGCCC ACAACTCTCT TAGTAGTCAT GGGAACACTG
               TTCTTTGAGG TCCAACCGGG TGTTGAGAGA ATCATCAGTA CCCTTGTGAC
                                    hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7801       GTGGCTTTGG TTGGTCTGTT CGTGCTGTTG GCTTTTCTTC AATATAGAAG
               CACCGAAACC AACCAGACAA GCACGACAAC CGAAAAGAAG TTATATCTTC
                                    hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7851       ACTTCGAAAA GGATATACAC CCCTAATGGA GACACATTTA AGCAGCAAGA
               TGAAGCTTTT CCTATATGTG GGGATTACCT CTGTGTAAAT TCGTCGTTCT
                         hTRP-2
               ~~~~~~~~~~~~~~~~~~~~~
    7901       GATACACAGA AGAAGCCTAG TTTTTTAATT AAGCATGCTC TAGAATCGAT
               CTATGTGTCT TCTTCGGATC AAAAAATTAA TTCGTACGAG ATCTTAGCTA
                                                         C5 Left Arm
                                                   ~~~~~~~~~~~~~~~~~~~
    7951       CCCGGGTTTT TATGACTAGT TAATCACGGC CGCTTATAAA GATCTAAAAT
               GGGCCCAAAA ATACTGATCA ATTAGTGCCG GCGAATATTT CTAGATTTTA
                                    C5 Left Arm
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8001       GCATAATTTC TAAATAATGA AAAAAAAGTA CATCATGAGC AACGCGTTAG
               CGTATTAAAG ATTTATTACT TTTTTTTCAT GTAGTACTCG TTGCGCAATC
                                    C5 Left Arm
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8051       TATATTTTAC AATGGAGATT AACGCTCTAT ACCGTTCTAT GTTTATTGAT
               ATATAAAATG TTACCTCTAA TTGCGAGATA TGGCAAGATA CAAATAACTA
                                    C5 Left Arm
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8101       TCAGATGATG TTTTAGAAAA GAAAGTTATT GAATATGAAA ACTTTAATGA
               AGTCTACTAC AAAATCTTTT CTTTCAATAA CTTATACTTT TGAAATTACT
                                    C5 Left Arm
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8151       AGATGAAGAT GACGACGATG ATTATTGTTG TAAATCTGTT TTAGATGAAG
               TCTACTTCTA CTGCTGCTAC TAATAACAAC ATTTAGACAA AATCTACTTC
                                    C5 Left Arm
               ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8201       AAGATGACGC GCTAAAGTAT ACTATGGTTA CAAAGTATAA GTCTATACTA
               TTCTACTGCG CGATTTCATA TGATACCAAT GTTTCATATT CAGATATGAT
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                         C5 Left Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8251  CTAATGGCGA CTTGTGCAAG AAGGTATAGT ATAGTGAAAA TGTTGTTAGA
          GATTACCGCT GAACACGTTC TTCCATATCA TATCACTTTT ACAACAATCT
                         C5 Left Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8301  TTATGATTAT GAAAAACCAA ATAAATCAGA TCCATATCTA AAGGTATCTC
          AATACTAATA CTTTTTGGTT TATTTAGTCT AGGTATAGAT TTCCATAGAG
                         C5 Left Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8351  CTTTGCACAT AATTTCATCT ATTCCTAGTT TAGAATACTT TTCATTATAT
          GAAACGTGTA TTAAAGTAGA TAAGGATCAA ATCTTATGAA AAGTAATATA
                         C5 Left Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8401  TTGTTTACAG CTGAAGACGA AAAAAATATA TCGATAATAG AAGATTATGT
          AACAAATGTC GACTTCTGCT TTTTTTATAT AGCTATTATC TTCTAATACA
                      C5 Left Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8451  TAACTCTGCT AATAAGATGA AATTGAATGA GTCTGTGACT GCAGCCAAGC
          ATTGAGACGA TTATTCTACT TTAACTTACT CAGACACTGA CGTCGGTTCG
    8501  TTGGCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT
          AACCGTGACC GGCAGCAAAA TGTTGCAGCA CTGACCCTTT TGGGACCGCA
    8551  TACCCAACTT AATCGCCTTG CAGCACATCC CCCTTTCGCC AGCTGGCGTA
          ATGGGTTGAA TTAGCGGAAC GTCGTGTAGG GGGAAAGCGG TCGACCGCAT
    8601  ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT GCGCAGCCTG
          TATCGCTTCT CCGGGCGTGG CTAGCGGGAA GGGTTGTCAA CGCGTCGGAC
    8651  AATGGCGAAT GGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG
          TTACCGCTTA CCGCGGACTA CGCCATAAAA GAGGAATGCG TAGACACGCC
    8701  TATTTCACAC CGCATATGGT GCACTCTCAG TACAATCTGC TCTGATGCCG
          ATAAAGTGTG GCGTATACCA CGTGAGAGTC ATGTTAGACG AGACTACGGC
    8751  CATAGTTAAG CCAGCCCCGA CACCCGCCAA CACCCGCTGA CGCGCCCTGA
          GTATCAATTC GGTCGGGGCT GTGGGCGGTT GTGGGCGACT GCGCGGGACT
    8801  CGGGCTTGTC TGCTCCCGGC ATCCGCTTAC AGACAAGCTG TGACCGTCTC
          GCCCGAACAG ACGAGGGCCG TAGGCGAATG TCTGTTCGAC ACTGGCAGAG
    8851  CGGGAGCTGC ATGTGTCAGA GGTTTTCACC GTCATCACCG AAACGCGCGA
          GCCCTCGACG TACACAGTCT CCAAAAGTGG CAGTAGTGGC TTTGCGCGCT
    8901  GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA
          CTGCTTTCCC GGAGCACTAT GCGGATAAAA ATATCCAATT ACAGTACTAT
    8951  ATAATGGTTT CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG
          TATTACCAAA GAATCTGCAG TCCACCGTGA AAAGCCCCTT TACACGCGCC
    9001  AACCCCTATT TGTTTATTTT TCTAAATACA TTCAAATATG TATCCGCTCA
          TTGGGGATAA ACAAATAAAA AGATTTATGT AAGTTTATAC ATAGGCGAGT
    9051  TGAGACAATA ACCCTGATAA ATGCTTCAAT AATATTGAAA AAGGAAGAGT
          ACTCTGTTAT TGGGACTATT TACGAAGTTA TTATAACTTT TTCCTTCTCA
                           Amp(R)
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    9101  ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT TTGCGGCATT
          TACTCATAAG TTGTAAAGGC ACAGCGGGAA TAAGGGAAAA AACGCCGTAA
                           Amp(R)
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    9151  TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG
          AACGGAAGGA CAAAAACGAG TGGGTCTTTG CGACCACTTT CATTTTCTAC
                           Amp(R)
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    9201  CTGAAGATCA GTTGGGTGCA CGAGTGGGTT ACATCGAACT GGATCTCAAC
          GACTTCTAGT CAACCCACGT GCTCACCCAA TGTAGCTTGA CCTAGAGTTG
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                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9251    AGCGGTAAGA TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT
        TCGCCATTCT AGGAACTCTC AAAAGCGGGG CTTCTTGCAA AAGGTTACTA
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9301    GAGCACTTTT AAAGTTCTGC TATGTGGCGC GGTATTATCC CGTATTGACG
        CTCGTGAAAA TTTCAAGACG ATACACCGCG CCATAATAGG GCATAACTGC
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9351    CCGGGCAAGA GCAACTCGGT CGCCGCATAC ACTATTCTCA GAATGACTTG
        GGCCCGTTCT CGTTGAGCCA GCGGCGTATG TGATAAGAGT CTTACTGAAC
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9401    GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG GCATGACAGT
        CAACTCATGA GTGGTCAGTG TCTTTTCGTA GAATGCCTAC CGTACTGTCA
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9451    AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA
        TTCTCTTAAT ACGTCACGAC GGTATTGGTA CTCACTATTG TGACGCCGGT
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9501    ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG
        TGAATGAAGA CTGTTGCTAG CCTCCTGGCT TCCTCGATTG GCGAAAAAAC
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9551    CACAACATGG GGGATCATGT AACTCGCCTT GATCGTTGGG AACCGGAGCT
        GTGTTGTACC CCCTAGTACA TTGAGCGGAA CTAGCAACCC TTGGCCTCGA
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9601    GAATGAAGCC ATACCAAACG ACGAGCGTGA CACCACGATG CCTGTAGCAA
        CTTACTTCGG TATGGTTTGC TGCTCGCACT GTGGTGCTAC GGACATCGTT
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9651    TGGCAACAAC GTTGCGCAAA CTATTAACTG GCGAACTACT TACTCTAGCT
        ACCGTTGTTG CAACGCGTTT GATAATTGAC CGCTTGATGA ATGAGATCGA
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9701    TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG TTGCAGGACC
        AGGGCCGTTG TTAATTATCT GACCTACCTC CGCCTATTTC AACGTCCTGG
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9751    ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG
        TGAAGACGCG AGCCGGGAAG GCCGACCGAC CAAATAACGA CTATTTAGAC
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9801    GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT
        CTCGGCCACT CGCACCCAGA GCGCCATAGT AACGTCGTGA CCCCGGTCTA
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9851    GGTAAGCCCT CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC
        CCATTCGGGA GGGCATAGCA TCAATAGATG TGCTGCCCCT CAGTCCGTTG
                               Amp(R)
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9901    TATGGATGAA CGAAATAGAC AGATCGCTGA GATAGGTGCC TCACTGATTA
        ATACCTACTT GCTTTATCTG TCTAGCGACT CTATCCACGG AGTGACTAAT
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          Amp(R)
          ~~~~~~~~~~~~~
 9951     AGCATTGGTA ACTGTCAGAC CAAGTTTACT CATATATACT TTAGATTGAT
          TCGTAACCAT TGACAGTCTG GTTCAAATGA GTATATATGA AATCTAACTA
10001     TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA TCCTTTTTGA
          AATTTTGAAG TAAAAATTAA ATTTTCCTAG ATCCACTTCT AGGAAAAACT
10051     TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT
          ATTAGAGTAC TGGTTTTAGG GAATTGCACT CAAAAGCAAG GTGACTCGCA
10101     CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG
          GTCTGGGGCA TCTTTTCTAG TTTCCTAGAA GAACTCTAGG AAAAAAAGAC
10151     CGCGTAATCT GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT
          GCGCATTAGA CGACGAACGT TTGTTTTTTT GGTGGCGATG GTCGCCACCA
10201     TTGTTTGCCG GATCAAGAGC TACCAACTCT TTTTCCGAAG GTAACTGGCT
          AACAAACGGC CTAGTTCTCG ATGGTTGAGA AAAAGGCTTC CATTGACCGA
10251     TCAGCAGAGC GCAGATACCA AATACTGTCC TTCTAGTGTA GCCGTAGTTA
          AGTCGTCTCG CGTCTATGGT TTATGACAGG AAGATCACAT CGGCATCAAT
10301     GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC TCGCTCTGCT
          CCGGTGGTGA AGTTCTTGAG ACATCGTGGC GGATGTATGG AGCGAGACGA
10351     AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG
          TTAGGACAAT GGTCACCGAC GACGGTCACC GCTATTCAGC ACAGAATGGC
10401     GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA
          CCAACCTGAG TTCTGCTATC AATGGCCTAT TCCGCGTCGC CAGCCCGACT
10451     ACGGGGGGTT CGTGCACACA GCCCAGCTTG GAGCGAACGA CCTACACCGA
          TGCCCCCCAA GCACGTGTGT CGGGTCGAAC CTCGCTTGCT GGATGTGGCT
10501     ACTGAGATAC CTACAGCGTG AGCTATGAGA AAGCGCCACG CTTCCCGAAG
          TGACTCTATG GATGTCGCAC TCGATACTCT TTCGCGGTGC GAAGGGCTTC
10551     GGAGAAAGGC GGACAGGTAT CCGGTAAGCG GCAGGGTCGG AACAGGAGAG
          CCTCTTTCCG CCTGTCCATA GGCCATTCGC CGTCCCAGCC TTGTCCTCTC
10601     CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT ATAGTCCTGT
          GCGTGCTCCC TCGAAGGTCC CCCTTTGCGG ACCATAGAAA TATCAGGACA
10651     CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG
          GCCCAAAGCG GTGGAGACTG AACTCGCAGC TAAAAACACT ACGAGCAGTC
10701     GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC
          CCCCCGCCTC GGATACCTTT TTGCGGTCGT TGCGCCGGAA AAATGCCAAG
10751     CTGGCCTTTT GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC
          GACCGGAAAA CGACCGGAAA ACGAGTGTAC AAGAAAGGAC GCAATAGGGG
10801     TGATTCTGTG GATAACCGTA TTACCGCCTT TGAGTGAGCT GATACCGCTC
          ACTAAGACAC CTATTGGCAT AATGGCGGAA ACTCACTCGA CTATGGCGAG
10851     GCCGCAGCCG AACGACCGAG CGCAGCGAGT CAGTGAGCGA GGAAGCGGAA
          CGGCGTCGGC TTGCTGGCTC GCGTCGCTCA GTCACTCGCT CCTTCGCCTT
10901     GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC CGATTCATTA
          CTCGCGGGTT ATGCGTTTGG CGGAGAGGGG CGCGCAACCG GCTAAGTAAT
10951     ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA
          TACGTCGACC GTGCTGTCCA AAGGGCTGAC CTTTCGCCCG TCACTCGCGT
11001     ACGCAATTAA TGTGAGTTAG CTCACTCATT AGGCACCCCA GGCTTTACAC
          TGCGTTAATT ACACTCAATC GAGTGAGTAA TCCGTGGGGT CCGAAATGTG
11051     TTTATGCTTC CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG GATAACAATT
          AAATACGAAG GCCGAGCATA CAACACACCT TAACACTCGC CTATTGTTAA
11101     TCACACAGGA AACAGCTATG ACCATGATTA CGAATTGAAT TGCGGCCGCA
          AGTGTGTCCT TTGTCGATAC TGGTACTAAT GCTTAACTTA ACGCCGGCGT
11151     ATTCTAAG
```

FIGURE 4

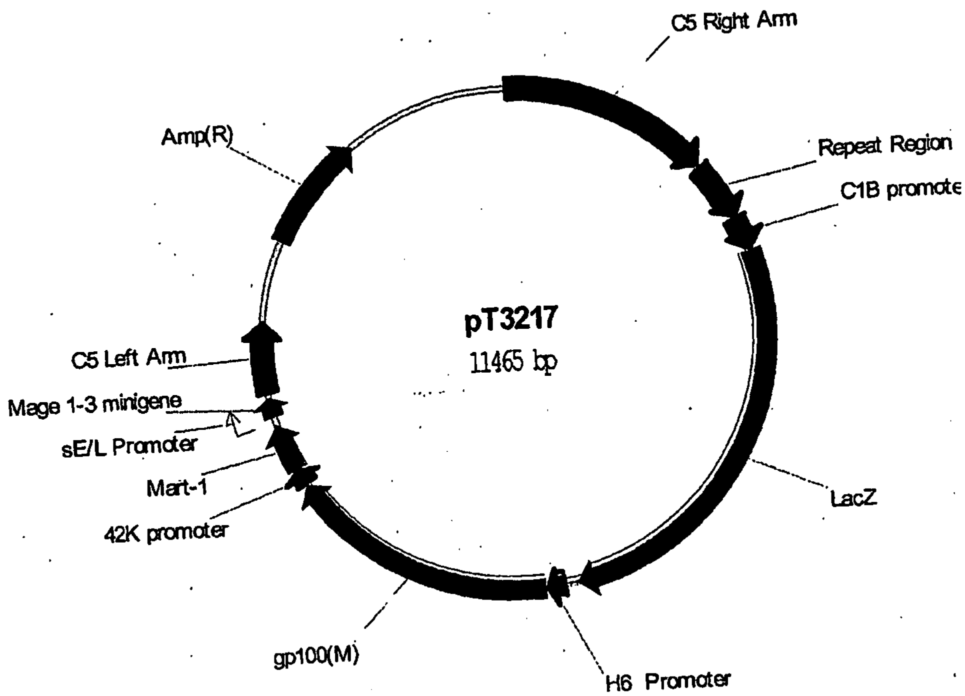
C5 Right Arm
Repeat Region
C1B promote
LacZ
H6 Promoter
gp100(M)
42K promoter
Mart-1
sE/L Promoter
Mage 1-3 minigene
C5 Left Arm
Amp(R)
pT3217
11465 bp

FIGURE 5

DNA Sequence of donor plasmid pT3217

```
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1 TGAATGTTAA ATGTTATACT TTGGATGAAG CTATAAATAT GCATTGGAAA
     ACTTACAATT TACAATATGA AACCTACTTC GATATTTATA CGTAACCTTT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
  51 AATAATCCAT TTAAAGAAAG GATTCAAATA CTACAAAACC TAAGCGATAA
     TTATTAGGTA AATTTCTTTC CTAAGTTTAT GATGTTTTGG ATTCGCTATT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 101 TATGTTAACT AAGCTTATTC TTAACGACGC TTTAAATATA CACAAATAAA
     ATACAATTGA TTCGAATAAG AATTGCTGCG AAATTTATAT GTGTTTATTT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 151 CATAATTTTT GTATAACCTA ACAAATAACT AAAACATAAA AATAATAAAA
     GTATTAAAAA CATATTGGAT TGTTTATTGA TTTTGTATTT TTATTATTTT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 201 GGAAATGTAA TATCGTAATT ATTTTACTCA GGAATGGGGT TAAATATTTA
     CCTTTACATT ATAGCATTAA TAAAATGAGT CCTTACCCCA ATTTATAAAT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 251 TATCACGTGT ATATCTATAC TGTTATCGTA TACTCTTTAC AATTACTATT
     ATAGTGCACA TATAGATATG ACAATAGCAT ATGAGAAATG TTAATGATAA
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 301 ACGAATATGC AAGAGATAAT AAGATTACGT ATTTAAGAGA ATCTTGTCAT
     TGCTTATACG TTCTCTATTA TTCTAATGCA TAAATTCTCT TAGAACAGTA
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 351 GATAATTGGG TACGACATAG TGATAAATGC TATTTCGCAT CGTTACATAA
     CTATTAACCC ATGCTGTATC ACTATTTACG ATAAAGCGTA GCAATGTATT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 401 AGTCAGTTGG AAAGATGGAT TTGACAGATG TAACTTAATA GGTGCAAAAA
     TCAGTCAACC TTTCTACCTA AACTGTCTAC ATTGAATTAT CCACGTTTTT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 451 TGTTAAATAA CAGCATTCTA TCGGAAGATA GGATACCAGT TATATTATAC
     ACAATTTATT GTCGTAAGAT AGCCTTCTAT CCTATGGTCA ATATAATATG
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 501 AAAAATCACT GGTTGGATAA AACAGATTCT GCAATATTCG TAAAAGATGA
     TTTTTAGTGA CCAACCTATT TTGTCTAAGA CGTTATAAGC ATTTTCTACT
                          C5 Right Arm
     ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 551 AGATTACTGC GAATTTGTAA ACTATGACAA TAAAAAGCCA TTTATCTCAA
     TCTAATGACG CTTAAACATT TGATACTGTT ATTTTTCGGT AAATAGAGTT
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                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 601    CGACATCGTG TAATTCTTCC ATGTTTTATG TATGTGTTTC AGATATTATG
        GCTGTAGCAC ATTAAGAAGG TACAAAATAC ATACACAAAG TCTATAATAC
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 651    AGATTACTAT AAACTTTTTG TATACTTATA TTCCGTAAAC TATATTAATC
        TCTAATGATA TTTGAAAAAC ATATGAATAT AAGGCATTTG ATATAATTAG
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 701    ATGAAGAAAA TGAAAAAGTA TAGAAGCTGT TCACGAGCGG TTGTTGAAAA
        TACTTCTTTT ACTTTTTCAT ATCTTCGACA AGTGCTCGCC AACAACTTTT
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 751    CAACAAAATT ATACATTCAA GATGGCTTAC ATATACGTCT GTGAGGCTAT
        GTTGTTTTAA TATGTAAGTT CTACCGAATG TATATGCAGA CACTCCGATA
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 801    CATGGATAAT GACAATGCAT CTCTAAATAG GTTTTTGGAC AATGGATTCG
        GTACCTATTA CTGTTACGTA GAGATTTATC CAAAAACCTG TTACCTAAGC
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 851    ACCCTAACAC GGAATATGGT ACTCTACAAT CTCCTCTTGA AATGGCTGTA
        TGGGATTGTG CCTTATACCA TGAGATGTTA GAGGAGAACT TTACCGACAT
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 901    ATGTTCAAGA ATACCGAGGC TATAAAAATC TTGATGAGGT ATGGAGCTAA
        TACAAGTTCT TATGGCTCCG ATATTTTTAG AACTACTCCA TACCTCGATT
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 951    ACCTGTAGTT ACTGAATGCA CAACTTCTTG TCTGCATGAT GCGGTGTTGA
        TGGACATCAA TGACTTACGT GTTGAAGAAC AGACGTACTA CGCCACAACT
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1001    GAGACGACTA CAAAATAGTG AAAGATCTGT TGAAGAATAA CTATGTAAAC
        CTCTGCTGAT GTTTTATCAC TTTCTAGACA ACTTCTTATT GATACATTTG
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1051    AATGTTCTTT ACAGCGGAGG CTTTACTCCT TTGTGTTTGG CAGCTTACCT
        TTACAAGAAA TGTCGCCTCC GAAATGAGGA AACACAAACC GTCGAATGGA
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1101    TAACAAAGTT AATTTGGTTA AACTTCTATT GGCTCATTCG GCGGATGTAG
        ATTGTTTCAA TTAAACCAAT TTGAAGATAA CCGAGTAAGC CGCCTACATC
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1151    ATATTTCAAA CACGGATCGG TTAACTCCTC TACATATAGC CGTATCAAAT
        TATAAAGTTT GTGCCTAGCC AATTGAGGAG ATGTATATCG GCATAGTTTA
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1201    AAAAATTTAA CAATGGTTAA ACTTCTATTG AACAAAGGTG CTGATACTGA
        TTTTTAAATT GTTACCAATT TGAAGATAAC TTGTTTCCAC GACTATGACT
                         C5 Right Arm
        ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
1251    CTTGCTGGAT AACATGGGAT GTACTCCTTT AATGATCGCT GTACAATCTG
        GAACGACCTA TTGTACCCTA CATGAGGAAA TTACTAGCGA CATGTTAGAC
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                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1301   GAAATATTGA AATATGTAGC ACACTACTTA AAAAAAATAA AATGTCCAGA
          CTTTATAACT TTATACATCG TGTGATGAAT TTTTTTTATT TTACAGGTCT
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1351   ACTGGGAAAA ATTGATCTTG CCAGCTGTAA TTCATGGTAG AAAAGAAGTG
          TGACCCTTTT TAACTAGAAC GGTCGACATT AAGTACCATC TTTTCTTCAC
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1401   CTCAGGCTAC TTTTCAACAA AGGAGCAGAT GTAAACTACA TCTTTGAAAG
          GAGTCCGATG AAAAGTTGTT TCCTCGTCTA CATTTGATGT AGAAACTTTC
                         C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1451   AAATGGAAAA TCATATACTG TTTTGGAATT GATTAAAGAA AGTTACTCTG
          TTTACCTTTT AGTATATGAC AAAACCTTAA CTAATTTCTT TCAATGAGAC
                  C5 Right Arm
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1501   AGACACAAAA GAGGTAGCTG AAGTGGTACT CTCAAAGGTA CGTGACTAAT
          TCTGTGTTTT CTCCATCGAC TTCACCATGA GAGTTTCCAT GCACTGATTA
                                     Repeat Region
                                 ~~~~~~~~~~~~~~~~~~~~~~~~~~
   1551   TAGCTATAAA AAGGATCGGG TTCTTTATTC TATACTTAAA AAGTGAAAAT
          ATCGATATTT TTCCTAGCCC AAGAAATAAG ATATGAATTT TTCACTTTTA
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1601   AAATACAAAG GTTCTTGAGG GTTGTGTTAA ATTGAAAGCG AGAAATAATC
          TTTATGTTTC CAAGAACTCC CAACACAATT TAACTTTCGC TCTTTATTAG
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1651   ATAAATTATT TCATTATCGC GATATCCGTT AAGTTTGTAT CGTAATCTGC
          TATTTAATAA AGTAATAGCG CTATAGGCAA TTCAAACATA GCATTAGACG
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1701   AGCCCCCACC ATGGATCTGG TGCTAAAAAG ATGCCTTCTT CATTTGGCTG
          TCGGGGGTGG TACCTAGACC ACGATTTTTC TACGGAAGAA GTAAACCGAC
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1751   TGATAGGTGC TTTGCTGGCT GTGGGGGCTA CAAAAGTACC CAGAAACCAG
          ACTATCCACG AAACGACCGA CACCCCCGAT GTTTTCATGG GTCTTTGGTC
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1801   GACTGGCTTG GTGTCTCAAG GCAACTCAGA ACCAAAGCCT GGAACAGGCA
          CTGACCGAAC CACAGAGTTC CGTTGAGTCT TGGTTTCGGA CCTTGTCCGT
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1851   GCTGTATCCA GAGTGGACAG AAGCCCAGAG ACTTGACTGC TGGAGAGGTG
          CGACATAGGT CTCACCTGTC TTCGGGTCTC TGAACTGACG ACCTCTCCAC
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1901   GTCAAGTGTC CCTCAAGGTC AGTAATGATG GGCCTACACT GATTGGTGCA
          CAGTTCACAG GGAGTTCCAG TCATTACTAC CCGGATGTGA CTAACCACGT
                        Repeat Region
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
   1951   AATGCCTCCT TCTCTATTGC CTTGAACTTC CCTGGAAGCC AAAAGGTATT
          TTACGGAGGA AGAGATAACG GAACTTGAAG GGACCTTCGG TTTTCCATAA
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      Repeat Region                 C1B promoter
      ~~~~~~~           ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2001  GCCAGATACT AGTTCTAGAG GATCATTATT TAACGTAAAC TAAATGGAAA
      CGGTCTATGA TCAAGATCTC CTAGTAATAA ATTGCATTTG ATTTACCTTT
                         C1B promoter
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2051  AGCTATTTAC AGGTACATAC GGTGTTTTTC TGGAATCAAA TGATTCTGAT
      TCGATAAATG TCCATGTATG CCACAAAAAG ACCTTAGTTT ACTAAGACTA
                         C1B promoter
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2101  TTTGAGGATT TTATCAATAC AATAATGACA GTGCTAACTG GTAAAAAAGA
      AAACTCCTAA AATAGTTATG TTATTACTGT CACGATTGAC CATTTTTTCT
                         C1B promoter
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2151  AAGCAAACAA TTATCATGGC TAACAATTTT TATTATATTT GTAGTATGCA
      TTCGTTTGTT AATAGTACCG ATTGTTAAAA ATAATATAAA CATCATACGT
                         C1B promoter
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2201  TAGTGGTCTT TACGTTTCTT TATTTAAAGT TAATGTGTTA AGATTAAATG
      ATCACCAGAA ATGCAAAGAA ATAAATTTCA ATTACACAAT TCTAATTTAC
      C1B promoter                    LacZ
      ~~~~~~~~~~~~~~      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2251  GAGTAATTGG ATCCCCCATC GATGGGGAAT TCACTGGCCG TCGTTTTACA
      CTCATTAACC TAGGGGGTAG CTACCCCTTA AGTGACCGGC AGCAAAATGT
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2301  ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCAG
      TGCAGCACTG ACCCTTTTGG GACCGCAATG GGTTGAATTA GCGGAACGTC
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2351  CACATCCCCC TTTCGCCAGC TGGCGTAATA GCGAAGAGGC CCGCACCGAT
      GTGTAGGGGG AAAGCGGTCG ACCGCATTAT CGCTTCTCCG GGCGTGGCTA
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2401  CGCCCTTCCC AACAGTTGCG CAGCCTGAAT GGCGAATGGC GCTTTGCCTG
      GCGGGAAGGG TTGTCAACGC GTCGGACTTA CCGCTTACCG CGAAACGGAC
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2451  GTTTCCGGCA CCAGAAGCGG TGCCGGAAAG CTGGCTGGAG TGCGATCTTC
      CAAAGGCCGT GGTCTTCGCC ACGGCCTTTC GACCGACCTC ACGCTAGAAG
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2501  CTGAGGCCGA TACTGTCGTC GTCCCCTCAA ACTGGCAGAT GCACGGTTAC
      GACTCCGGCT ATGACAGCAG CAGGGGAGTT TGACCGTCTA CGTGCCAATG
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2551  GATGCGCCCA TCTACACCAA CGTGACCTAT CCCATTACGG TCAATCCGCC
      CTACGCGGGT AGATGTGGTT GCACTGGATA GGGTAATGCC AGTTAGGCGG
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2601  GTTTGTTCCC ACGGAGAATC CGACGGGTTG TTACTCGCTC ACATTTAATG
      CAAACAAGGG TGCCTCTTAG GCTGCCCAAC AATGAGCGAG TGTAAATTAC
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
2651  TTGATGAAAG CTGGCTACAG GAAGGCCAGA CGCGAATTAT TTTGATGGC
      AACTACTTTC GACCGATGTC CTTCCGGTCT GCGCTTAATA AAAACTACCG
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                               LacZ
2701  GTTAACTCGG CGTTTCATCT GTGGTGCAAC GGGCGCTGGG TCGGTTACGG
      CAATTGAGCC GCAAAGTAGA CACCACGTTG CCCGCGACCC AGCCAATGCC
                               LacZ
2751  CCAGGACAGT CGTTTGCCGT CTGAATTTGA CCTGAGCGCA TTTTTACGCG
      GGTCCTGTCA GCAAACGGCA GACTTAAACT GGACTCGCGT AAAAATGCGC
                               LacZ
2801  CCGGAGAAAA CCGCCTCGCG GTGATGGTGC TGCGCTGGAG TGACGGCAGT
      GGCCTCTTTT GGCGGAGCGC CACTACCACG ACGCGACCTC ACTGCCGTCA
                               LacZ
2851  TATCTGGAAG ATCAGGATAT GTGGCGGATG AGCGGCATTT TCCGTGACGT
      ATAGACCTTC TAGTCCTATA CACCGCCTAC TCGCCGTAAA AGGCACTGCA
                               LacZ
2901  CTCGTTGCTG CATAAACCGA CTACACAAAT CAGCGATTTC CATGTTGCCA
      GAGCAACGAC GTATTTGGCT GATGTGTTTA GTCGCTAAAG GTACAACGGT
                               LacZ
2951  CTCGCTTTAA TGATGATTTC AGCCGCGCTG TACTGGAGGC TGAAGTTCAG
      GAGCGAAATT ACTACTAAAG TCGGCGCGAC ATGACCTCCG ACTTCAAGTC
                               LacZ
3001  ATGTGCGGCG AGTTGCGTGA CTACCTACGG GTAACAGTTT CTTTATGGCA
      TACACGCCGC TCAACGCACT GATGGATGCC CATTGTCAAA GAAATACCGT
                               LacZ
3051  GGGTGAAACG CAGGTCGCCA GCGGCACCGC GCCTTTCGGC GGTGAAATTA
      CCCACTTTGC GTCCAGCGGT CGCCGTGGCG CGGAAAGCCG CCACTTTAAT
                               LacZ
3101  TCGATGAGCG TGGTGGTTAT GCCGATCGCG TCACACTACG TCTGAACGTC
      AGCTACTCGC ACCACCAATA CGGCTAGCGC AGTGTGATGC AGACTTGCAG
                               LacZ
3151  GAAAACCCGA AACTGTGGAG CGCCGAAATC CCGAATCTCT ATCGTGCGGT
      CTTTTGGGCT TTGACACCTC GCGGCTTTAG GGCTTAGAGA TAGCACGCCA
                               LacZ
3201  GGTTGAACTG CACACCGCCG ACGGCACGCT GATTGAAGCA GAAGCCTGCG
      CCAACTTGAC GTGTGGCGGC TGCCGTGCGA CTAACTTCGT CTTCGGACGC
                               LacZ
3251  ATGTCGGTTT CCGCGAGGTG CGGATTGAAA ATGGTCTGCT GCTGCTGAAC
      TACAGCCAAA GGCGCTCCAC GCCTAACTTT TACCAGACGA CGACGACTTG
                               LacZ
3301  GGCAAGCCGT TGCTGATTCG AGGCGTTAAC CGTCACGAGC ATCATCCTCT
      CCGTTCGGCA ACGACTAAGC TCCGCAATTG GCAGTGCTCG TAGTAGGAGA
                               LacZ
3351  GCATGGTCAG GTCATGGATG AGCAGACGAT GGTGCAGGAT ATCCTGCTGA
      CGTACCAGTC CAGTACCTAC TCGTCTGCTA CCACGTCCTA TAGGACGACT
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                                        LacZ
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    3401    TGAAGCAGAA CAACTTTAAC GCCGTGCGCT GTTCGCATTA TCCGAACCAT
            ACTTCGTCTT GTTGAAATTG CGGCACGCGA CAAGCGTAAT AGGCTTGGTA
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3451    CCGCTGTGGT ACACGCTGTG CGACCGCTAC GGCCTGTATG TGGTGGATGA
            GGCGACACCA TGTGCGACAC GCTGGCGATG CCGGACATAC ACCACCTACT
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3501    AGCCAATATT GAAACCCACG GCATGGTGCC AATGAATCGT CTGACCGATG
            TCGGTTATAA CTTTGGGTGC CGTACCACGG TTACTTAGCA GACTGGCTAC
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3551    ATCCGCGCTG GCTACCGGCG ATGAGCGAAC GCGTAACGCG AATGGTGCAG
            TAGGCGCGAC CGATGGCCGC TACTCGCTTG CGCATTGCGC TTACCACGTC
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3601    CGCGATCGTA ATCACCCGAG TGTGATCATC TGGTCGCTGG GGAATGAATC
            GCGCTAGCAT TAGTGGGCTC ACACTAGTAG ACCAGCGACC CCTTACTTAG
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3651    AGGCCACGGC GCTAATCACG ACGCGCTGTA TCGCTGGATC AAATCTGTCG
            TCCGGTGCCG CGATTAGTGC TGCGCGACAT AGCGACCTAG TTTAGACAGC
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3701    ATCCTTCCCG CCCGGTGCAG TATGAAGGCG GCGGAGCCGA CACCACGGCC
            TAGGAAGGGC GGGCCACGTC ATACTTCCGC CGCCTCGGCT GTGGTGCCGG
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3751    ACCGATATTA TTTGCCCGAT GTACGCGCGC GTGGATGAAG ACCAGCCCTT
            TGGCTATAAT AAACGGGCTA CATGCGCGCG CACCTACTTC TGGTCGGGAA
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3801    CCCGGCTGTG CCGAAATGGT CCATCAAAAA ATGGCTTTCG CTACCTGGAG
            GGGCCGACAC GGCTTTACCA GGTAGTTTTT TACCGAAAGC GATGGACCTC
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3851    AGACGCGCCC GCTGATCCTT TGCGAATACG CCCACGCGAT GGGTAACAGT
            TCTGCGCGGG CGACTAGGAA ACGCTTATGC GGGTGCGCTA CCCATTGTCA
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3901    CTTGGCGGTT TCGCTAAATA CTGGCAGGCG TTTCGTCAGT ATCCCCGTTT
            GAACCGCCAA AGCGATTTAT GACCGTCCGC AAAGCAGTCA TAGGGGCAAA
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    3951    ACAGGGCGGC TTCGTCTGGG ACTGGGTGGA TCAGTCGCTG ATTAAATATG
            TGTCCCGCCG AAGCAGACCC TGACCCACCT AGTCAGCGAC TAATTTATAC
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4001    ATGAAAACGG CAACCCGTGG TCGGCTTACG GCGGTGATTT TGGCGATACG
            TACTTTTGCC GTTGGGCACC AGCCGAATGC CGCCACTAAA ACCGCTATGC
                                        LacZ
            ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    4051    CCGAACGATC GCCAGTTCTG TATGAACGGT CTGGTCTTTG CCGACCGCAC
            GGCTTGCTAG CGGTCAAGAC ATACTTGCCA GACCAGAAAC GGCTGGCGTG
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                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4101     GCCGCATCCA GCGCTGACGG AAGCAAAACA CCAGCAGCAG TTTTTCCAGT
                CGGCGTAGGT CGCGACTGCC TTCGTTTTGT GGTCGTCGTC AAAAAGGTCA
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4151     TCCGTTTATC CGGGCAAACC ATCGAAGTGA CCAGCGAATA CCTGTTCCGT
                AGGCAAATAG GCCCGTTTGG TAGCTTCACT GGTCGCTTAT GGACAAGGCA
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4201     CATAGCGATA ACGAGCTCCT GCACTGGATG GTGGCGCTGG ATGGTAAGCC
                GTATCGCTAT TGCTCGAGGA CGTGACCTAC CACCGCGACC TACCATTCGG
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4251     GCTGGCAAGC GGTGAAGTGC CTCTGGATGT CGCTCCACAA GGTAAACAGT
                CGACCGTTCG CCACTTCACG GAGACCTACA GCGAGGTGTT CCATTTGTCA
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4301     TGATTGAACT GCCTGAACTA CCGCAGCCGG AGAGCGCCGG GCAACTCTGG
                ACTAACTTGA CGGACTTGAT GGCGTCGGCC TCTCGCGGCC CGTTGAGACC
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4351     CTCACAGTAC GCGTAGTGCA ACCGAACGCG ACCGCATGGT CAGAAGCCGG
                GAGTGTCATG CGCATCACGT TGGCTTGCGC TGGCGTACCA GTCTTCGGCC
                                        LacZ
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       4401     GCACATCAGC GCCTGGCAGC AGTGGCGTCT GGCGGAAAAC CTCAGTGTGA
                CGTGTAGTCG CGGACCGTCG TCACCGCAGA CCGCCTTTTG GAGTCACACT
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4451     CGCTCCCCGC CGCGTCCCAC GCCATCCCGC ATCTGACCAC CAGCGAAATG
                GCGAGGGGCG GCGCAGGGTG CGGTAGGGCG TAGACTGGTG GTCGCTTTAC
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4501     GATTTTTGCA TCGAGCTGGG TAATAAGCGT TGGCAATTTA ACCGCCAGTC
                CTAAAAACGT AGCTCGACCC ATTATTCGCA ACCGTTAAAT TGGCGGTCAG
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4551     AGGCTTTCTT TCACAGATGT GGATTGGCGA TAAAAAACAA CTGCTGACGC
                TCCGAAAGAA AGTGTCTACA CCTAACCGCT ATTTTTTGTT GACGACTGCG
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4601     CGCTGCGCGA TCAGTTCACC CGTGCACCGC TGGATAACGA CATTGGCGTA
                GCGACGCGCT AGTCAAGTGG GCACGTGGCG ACCTATTGCT GTAACCGCAT
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4651     AGTGAAGCGA CCCGCATTGA CCCTAACGCC TGGGTCGAAC GCTGGAAGGC
                TCACTTCGCT GGGCGTAACT GGGATTGCGG ACCCAGCTTG CGACCTTCCG
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4701     GGCGGGCCAT TACCAGGCCG AAGCAGCGTT GTTGCAGTGC ACGGCAGATA
                CCGCCCGGTA ATGGTCCGGC TTCGTCGCAA CAACGTCACG TGCCGTCTAT
                                        LacZ
                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
       4751     CACTTGCTGA TGCGGTGCTG ATTACGACCG CTCACGCGTG GCAGCATCAG
                GTGAACGACT ACGCCACGAC TAATGCTGGC GAGTGCGCAC CGTCGTAGTC
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                              LacZ
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4801  GGGAAAACCT TATTTATCAG CCGGAAAACC TACCGGATTG ATGGTAGTGG
      CCCTTTTGGA ATAAATAGTC GGCCTTTTGG ATGGCCTAAC TACCATCACC
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4851  TCAAATGGCG ATTACCGTTG ATGTTGAAGT GGCGAGCGAT ACACCGCATC
      AGTTTACCGC TAATGGCAAC TACAACTTCA CCGCTCGCTA TGTGGCGTAG
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4901  CGGCGCGGAT TGGCCTGAAC TGCCAGCTGG CGCAGGTAGC AGAGCGGGTA
      GCCGCGCCTA ACCGGACTTG ACGGTCGACC GCGTCCATCG TCTCGCCCAT
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
4951  AACTGGCTCG GATTAGGGCC GCAAGAAAAC TATCCCGACC GCCTTACTGC
      TTGACCGAGC CTAATCCCGG CGTTCTTTTG ATAGGGCTGG CGGAATGACG
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5001  CGCCTGTTTT GACCGCTGGG ATCTGCCATT GTCAGACATG TATACCCCGT
      GCGGACAAAA CTGGCGACCC TAGACGGTAA CAGTCTGTAC ATATGGGGCA
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5051  ACGTCTTCCC GAGCGAAAAC GGTCTGCGCT GCGGGACGCG CGAATTGAAT
      TGCAGAAGGG CTCGCTTTTG CCAGACGCGA CGCCCTGCGC GCTTAACTTA
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5101  TATGGCCCAC ACCAGTGGCG CGGCGACTTC CAGTTCAACA TCAGCCGGTA
      ATACCGGGTG TGGTCACCGC GCCGCTGAAG GTCAAGTTGT AGTCGGCCAT
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5151  CAGTCAACAG CAATTGATGG AAACCAGCCA TTCGCCATCT GCTGCACGCG
      GTCAGTTGTC GTTAACTACC TTTGGTCGGT AAGCGGTAGA CGACGTGCGC
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5201  GAAGAGGCAC ATGGCTGAAT ATCGACGGTT TCCATATGGG GATTGGTGGC
      CTTCTCCGTG TACCGACTTA TAGCTGCCAA AGGTATACCC CTAACCACCG
                              LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5251  GACGACTCCT GGAGCCCGTC AGTATCGGCG GAATTCCAGC TGAGCGCCGG
      CTGCTGAGGA CCTCGGGCAG TCATAGCCGC CTTAAGGTCG ACTCGCGGCC
                    LacZ
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5301  TCGCTACCAT TACCAGTTGG TCTGGTGTCA AAAATAATAA TAACCGGGCA
      AGCGATGGTA ATGGTCAACC AGACCACAGT TTTTATTATT ATTGGCCCGT
5351  GGGGGGATCC GGAGCTTATC GCAGATCAAT TCGATATCAA GCTTATCGAT
      CCCCCCTAGG CCTCGAATAG CGTCTAGTTA AGCTATAGTT CGAATAGCTA
                                                H6  Promoter
                                             ~~~~~~~~~~~~~~~~~~
5401  ACCGTCGACC TCGAGTCTAG AATCGATCCC GGGTTCTTTA TTCTATACTT
      TGGCAGCTGG AGCTCAGATC TTAGCTAGGG CCCAAGAAAT AAGATATGAA
                          H6  Promoter
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5451  AAAAAGTGAA AATAAATACA AAGGTTCTTG AGGGTTGTGT TAAATTGAAA
      TTTTTCACTT TTATTTATGT TTCCAAGAAC TCCCAACACA ATTTAACTTT
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      H6  Promoter
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5501  GCGAGAAATA ATCATAAATT ATTTCATTAT CGCGATATCC GTTAAGTTTG
      CGCTCTTTAT TAGTATTTAA TAAAGTAATA GCGCTATAGG CAATTCAAAC
      H6  Promoter                      gp100(M)
      ~~~~~~~                    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5551  TATCGTAATC TGCAGCCCCC ACCATGGATC TGGTGCTAAA AAGATGCCTT
      ATAGCATTAG ACGTCGGGGG TGGTACCTAG ACCACGATTT TTCTACGGAA
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5601  CTTCATTTGG CTGTGATAGG TGCTTTGCTG GCTGTGGGGG CTACAAAAGT
      GAAGTAAACC GACACTATCC ACGAAACGAC CGACACCCCC GATGTTTTCA
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5651  ACCCAGAAAC CAGGACTGGC TTGGTGTCTC AAGGCAACTC AGAACCAAAG
      TGGGTCTTTG GTCCTGACCG AACCACAGAG TTCCGTTGAG TCTTGGTTTC
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5701  CCTGGAACAG GCAGCTGTAT CCAGAGTGGA CAGAAGCCCA GAGACTTGAC
      GGACCTTGTC CGTCGACATA GGTCTCACCT GTCTTCGGGT CTCTGAACTG
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5751  TGCTGGAGAG GTGGTCAAGT GTCCCTCAAG GTCAGTAATG ATGGGCCTAC
      ACGACCTCTC CACCAGTTCA CAGGGAGTTC CAGTCATTAC TACCCGGATG
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5801  ACTGATTGGT GCAAATGCCT CCTTCTCTAT TGCCTTGAAC TTCCCTGGAA
      TGACTAACCA CGTTTACGGA GGAAGAGATA ACGGAACTTG AAGGGACCTT
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5851  GCCAAAAGGT ATTGCCAGAT GGGCAGGTTA TCTGGGTCAA CAATACCATC
      CGGTTTTCCA TAACGGTCTA CCCGTCCAAT AGACCCAGTT GTTATGGTAG
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5901  ATCAATGGGA GCCAGGTGTG GGGAGGACAG CCAGTGTATC CCCAGGAAAC
      TAGTTACCCT CGGTCCACAC CCCTCCTGTC GGTCACATAG GGGTCCTTTG
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
5951  TGACGATGCC TGCATCTTCC CTGATGGTGG ACCTTGCCCA TCTGGCTCTT
      ACTGCTACGG ACGTAGAAGG GACTACCACC TGGAACGGGT AGACCGAGAA
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6001  GGTCTCAGAA GAGAAGCTTT GTTTATGTCT GGAAGACCTG GGGCCAATAC
      CCAGAGTCTT CTCTTCGAAA CAAATACAGA CCTTCTGGAC CCCGGTTATG
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6051  TGGCAAGTTC TAGGGGGCCC AGTGTCTGGG CTGAGCATTG GGACAGGCAG
      ACCGTTCAAG ATCCCCCGGG TCACAGACCC GACTCGTAAC CCTGTCCGTC
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6101  GGCAATGCTG GGCACACACA CGATGGAAGT GACTGTCTAC CATCGCCGGG
      CCGTTACGAC CCGTGTGTGT GCTACCTTCA CTGACAGATG GTAGCGGCCC
                            gp100(M)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6151  GATCCCGGAG CTATGTGCCT CTTGCTCATT CCAGCTCAGC CTTCACCATT
      CTAGGGCCTC GATACACGGA GAACGAGTAA GGTCGAGTCG GAAGTGGTAA
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                                 gp100(M)
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6201      ATGGACCAGG TGCCTTTCTC CGTGAGCGTG TCCCAGTTGC GGGCCTTGGA
          TACCTGGTCC ACGGAAAGAG GCACTCGCAC AGGGTCAACG CCCGGAACCT
                                 gp100(M)
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6251      TGGAGGGAAC AAGCACTTCC TGAGAAATCA GCCTCTGACC TTTGCCCTCC
          ACCTCCCTTG TTCGTGAAGG ACTCTTTAGT CGGAGACTGG AAACGGGAGG
                                 gp100(M)
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6301      AGCTCCATGA CCCCAGTGGC TATCTGGCTG AAGCTGACCT CTCCTACACC
          TCGAGGTACT GGGGTCACCG ATAGACCGAC TTCGACTGGA GAGGATGTGG
                                 gp100(M)
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6351      TGGGACTTTG GAGACAGTAG TGGAACCCTG ATCTCTCGGG CACTTGTGGT
          ACCCTGAAAC CTCTGTCATC ACCTTGGGAC TAGAGAGCCC GTGAACACCA
                                 gp100(M)
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6401      CACTCATACT TACCTGGAGC CTGGCCCAGT CACTGTTCAG GTGGTCCTGC
          GTGAGTATGA ATGGACCTCG GACCGGGTCA GTGACAAGTC CACCAGGACG
                                 gp100(M)
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6451      AGGCTGCCAT TCCTCTCACC TCCTGTGGCT CCTCCCCAGT TCCAGGCACC
          TCCGACGGTA AGGAGAGTGG AGGACACCGA GGAGGGGTCA AGGTCCGTGG
                                 gp100(M)
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6501      ACAGATGGGC ACAGGCCAAC TGCAGAGGCC CCTAACACCA CAGCTGGCCA
          TGTCTACCCG TGTCCGGTTG ACGTCTCCGG GGATTGTGGT GTCGACCGGT
                                 gp100(M)
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6551      AGTGCCTACT ACAGAAGTTG TGGGTACTAC ACCTGGTCAG GCGCCAACTG
          TCACGGATGA TGTCTTCAAC ACCCATGATG TGGACCAGTC CGCGGTTGAC
                                 gp100(M)
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6601      CAGAGCCCTC TGGAACCACA TCTGTGCAGG TGCCAACCAC TGAAGTCATA
          GTCTCGGGAG ACCTTGGTGT AGACACGTCC ACGGTTGGTG ACTTCAGTAT
                                 gp100(M)
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6651      AGCACTGCAC CTGTGCAGAT GCCAACTGCA GAGAGCACAG GTATGACACC
          TCGTGACGTG GACACGTCTA CGGTTGACGT CTCTCGTGTC CATACTGTGG
                                 gp100(M)
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6701      TGAGAAGGTG CCAGTTTCAG AGGTCATGGG TACCACACTG GCAGAGATGT
          ACTCTTCCAC GGTCAAAGTC TCCAGTACCC ATGGTGTGAC CGTCTCTACA
                                 gp100(M)
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
6751      CAACTCCAGA GGCTACAGGT ATGACACCTG CAGAGGTATC AATTGTGGTG
          GTTGAGGTCT CCGATGTCCA TACTGTGGAC GTCTCCATAG TTAACACCAC
                                 gp100(M)
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6801      CTTTCTGGAA CCACAGCTGC ACAGGTAACA ACTACAGAGT GGGTGGAGAC
          GAAAGACCTT GGTGTCGACG TGTCCATTGT TGATGTCTCA CCCACCTCTG
                                 gp100(M)
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6851      CACAGCTAGA GAGCTACCTA TCCCTGAGCC TGAAGGTCCA GATGCCAGCT
          GTGTCGATCT CTCGATGGAT AGGGACTCGG ACTTCCAGGT CTACGGTCGA
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                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 6901  CAATCATGTC TACGGAAAGT ATTACAGGTT CCCTGGGCCC CCTGCTGGAT
       GTTAGTACAG ATGCCTTTCA TAATGTCCAA GGGACCCGGG GGACGACCTA
                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 6951  GGTACAGCCA CCTTAAGGCT GGTGAAGAGA CAAGTCCCCC TGGATTGTGT
       CCATGTCGGT GGAATTCCGA CCACTTCTCT GTTCAGGGGG ACCTAACACA
                       gp100(M)
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 7001  TCTGTATCGA TATGGTTCCT TTTCCGTCAC CCTGGACATT GTCCAGGGTA
       AGACATAGCT ATACCAAGGA AAAGGCAGTG GGACCTGTAA CAGGTCCCAT
                       gp100(M)
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 7051  TTGAAAGTGC CGAGATCCTG CAGGCTGTGC CGTCCGGTGA GGGGGATGCA
       AACTTTCACG GCTCTAGGAC GTCCGACACG GCAGGCCACT CCCCCTACGT
                       gp100(M)
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 7101  TTTGAGCTGA CTGTGTCCTG CCAAGGCGGG CTGCCCAAGG AAGCCTGCAT
       AAACTCGACT GACACAGGAC GGTTCCGCCC GACGGGTTCC TTCGGACGTA
                       gp100(M)
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 7151  GGAGATCTCA TCGCCAGGGT GCCAGCCCCC TGCCCAGCGG CTGTGCCAGC
       CCTCTAGAGT AGCGGTCCCA CGGTCGGGGG ACGGGTCGCC GACACGGTCG
                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 7201  CTGTGCTACC CAGCCCAGCC TGCCAGCTGG TTCTGCACCA GATACTGAAG
       GACACGATGG GTCGGGTCGG ACGGTCGACC AAGACGTGGT CTATGACTTC
                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 7251  GGTGGCTCGG GGACATACTG CCTCAATGTG TCTCTGGCTG ATACCAACAG
       CCACCGAGCC CCTGTATGAC GGAGTTACAC AGAGACCGAC TATGGTTGTC
                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 7301  CCTGGCAGTG GTCAGCACCC AGCTTATCAT GCCTGGTCAA GAAGCAGGCC
       GGACCGTCAC CAGTCGTGGG TCGAATAGTA CGGACCAGTT CTTCGTCCGG
                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 7351  TTGGGCAGGT TCCGCTGATC GTGGGCATCT TGCTGGTGTT GATGGCTGTG
       AACCCGTCCA AGGCGACTAG CACCCGTAGA ACGACCACAA CTACCGACAC
                       gp100(M)
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 7401  GTCCTTGCAT CTCTGATATA TAGGCGCAGA CTTATGAAGC AAGACTTCTC
       CAGGAACGTA GAGACTATAT ATCCGCGTCT GAATACTTCG TTCTGAAGAG
                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 7451  CGTACCCCAG TTGCCACATA GCAGCAGTCA CTGGCTGCGT CTACCCCGCA
       GCATGGGGTC AACGGTGTAT CGTCGTCAGT GACCGACGCA GATGGGGCGT
                       gp100(M)
       ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 7501  TCTTCTGCTC TTGTCCCATT GGTGAGAACA GCCCCCTCCT CAGTGGGCAG
       AGAAGACGAG AACAGGGTAA CCACTCTTGT CGGGGGAGGA GTCACCCGTC
       gp100(M)                      42K promoter
       ~~~~~~~~~                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 7551  CAGGTCTGAT TTTTATTCTA GTTCAAAAAA ATATAAATGA TTCACCATCT
       GTCCAGACTA AAAATAAGAT CAAGTTTTTT TATATTTACT AAGTGGTAGA
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                           42K promoter
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    7601  GATAGAAAAA AAATTTATTG GGAGAATATG ATAATATTTT GGGATTTCAA
          CTATCTTTTT TTTAAATAAC CCTCTTATAC TATTATAAAA CCCTAAAGTT
                 42K promoter                         Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~         ~~~~~~~~~~~~
    7651  AATTGAAAAT ATATAATTAC AATATAAATC TAGACCACCA TGCCAAGAGA
          TTAACTTTTA TATATTAATG TTATATTTAG ATCTGGTGGT ACGGTTCTCT
                                    Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7701  AGATGCTCAC TTCATCTATG GTTACCCCAA GAAGGGGCAC GGCCACTCTT
          TCTACGAGTG AAGTAGATAC CAATGGGGTT CTTCCCCGTG CCGGTGAGAA
                                    Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7751  ACACCACGGC TGAAGAGGCC GCTGGGATCG GCATCCTGAC AGTGATCCTG
          TGTGGTGCCG ACTTCTCCGG CGACCCTAGC CGTAGGACTG TCACTAGGAC
                                    Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7801  GGAGTCTTAC TGCTCATCGG CTGTTGGTAT TGTAGAAGAC GAAATGGATA
          CCTCAGAATG ACGAGTAGCC GACAACCATA ACATCTTCTG CTTTACCTAT
                                    Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7851  CAGAGCCTTG ATGGATAAAA GTCTTCATGT TGGCACTCAA TGTGCCTTAA
          GTCTCGGAAC TACCTATTTT CAGAAGTACA ACCGTGAGTT ACACGGAATT
                                    Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7901  CAAGAAGATG CCCACAAGAA GGGTTTGATC ATCGGGACAG CAAAGTGTCT
          GTTCTTCTAC GGGTGTTCTT CCCAAACTAG TAGCCCTGTC GTTTCACAGA
                                    Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    7951  CTTCAAGAGA AAAACTGTGA ACCTGTGGTT CCCAATGCTC CACCTGCTTA
          GAAGTTCTCT TTTTGACACT TGGACACCAA GGGTTACGAG GTGGACGAAT
                                  Mart-1
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8001  TGAGAAACTC TCTGCAGAAC AGTCACCACC ACCTTATTCA CCTTAATCTA
          ACTCTTTGAG AGACGTCTTG TCAGTGGTGG TGGAATAAGT GGAATTAGAT
                                               sE/L Promoter
                                ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8051  GAGTCGACCT GCAGGCATGC AAAAATTGAA ATTTTATTTT TTTTTTTTGG
          CTCAGCTGGA CGTCCGTACG TTTTTAACTT TAAAATAAAA AAAAAAAACC
          sE/L Promoter
          ~~~~~~~~~~~

                                Mage 1-3 minigene
                      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8101  AATATAAATA ATGGAGTCCT TGCAGCTGGT CTTTGGCATT GACGTGAAGG
          TTATATTTAT TACCTCAGGA ACGTCGACCA GAAACCGTAA CTGCACTTCC
                            Mage 1-3 minigene
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8151  AAGCAGACCC CACCGGCCAC TCCTATGTCC TTGTCACCTG CCTAGGTCTC
          TTCGTCTGGG GTGGCCGGTG AGGATACAGG AACAGTGGAC GGATCCAGAG
                            Mage 1-3 minigene
          ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    8201  TCCTATGATG GCAATAAGCG TAAAGAAGTG GACCCCATCG GCCACTTGTA
          AGGATACTAC CGTTATTCGC ATTTCTTCAC CTGGGGTAGC CGGTGAACAT
```

```
      Mage 1-3 minigene                                C5 Left Arm
          ~~~~                                            ~~~~~~~
8251  CTAGTTTTTA TCCCGGGTTT TTATGACTAG TTAATCACGG CCGCTTATAA
      GATCAAAAAT AGGGCCCAAA AATACTGATC AATTAGTGCC GGCGAATATT
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8301  AGATCTAAAA TGCATAATTT CTAAATAATG AAAAAAAAGT ACATCATGAG
      TCTAGATTTT ACGTATTAAA GATTTATTAC TTTTTTTTCA TGTAGTACTC
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8351  CAACGCGTTA GTATATTTTA CAATGGAGAT TAACGCTCTA TACCGTTCTA
      GTTGCGCAAT CATATAAAAT GTTACCTCTA ATTGCGAGAT ATGGCAAGAT
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8401  TGTTTATTGA TTCAGATGAT GTTTTAGAAA AGAAAGTTAT TGAATATGAA
      ACAAATAACT AAGTCTACTA CAAAATCTTT TCTTTCAATA ACTTATACTT
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8451  AACTTTAATG AAGATGAAGA TGACGACGAT GATTATTGTT GTAAATCTGT
      TTGAAATTAC TTCTACTTCT ACTGCTGCTA CTAATAACAA CATTTAGACA
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8501  TTTAGATGAA GAAGATGACG CGCTAAAGTA TACTATGGTT ACAAAGTATA
      AAATCTACTT CTTCTACTGC GCGATTTCAT ATGATACCAA TGTTTCATAT
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8551  AGTCTATACT ACTAATGGCG ACTTGTGCAA GAAGGTATAG TATAGTGAAA
      TCAGATATGA TGATTACCGC TGAACACGTT CTTCCATATC ATATCACTTT
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8601  ATGTTGTTAG ATTATGATTA TGAAAAACCA AATAAATCAG ATCCATATCT
      TACAACAATC TAATACTAAT ACTTTTTGGT TTATTTAGTC TAGGTATAGA
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8651  AAAGGTATCT CCTTTGCACA TAATTTCATC TATTCCTAGT TTAGAATACT
      TTTCCATAGA GGAAACGTGT ATTAAAGTAG ATAAGGATCA AATCTTATGA
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8701  TTTCATTATA TTTGTTTACA GCTGAAGACG AAAAAAATAT ATCGATAATA
      AAAGTAATAT AAACAAATGT CGACTTCTGC TTTTTTTATA TAGCTATTAT
                          C5 Left Arm
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
8751  GAAGATTATG TTAACTCTGC TAATAAGATG AAATTGAATG AGTCTGTGAC
      CTTCTAATAC AATTGAGACG ATTATTCTAC TTTAACTTAC TCAGACACTG
      C5 Left Arm
      ~~~~~
8801  TGCAGCCAAG CTTGGCACTG GCCGTCGTTT TACAACGTCG TGACTGGGAA
      ACGTCGGTTC GAACCGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCTT
8851  AACCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTTCGC
      TTGGGACCGC AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG
8901  CAGCTGGCGT AATAGCGAAG AGGCCCGCAC CGATCGCCCT TCCCAACAGT
      GTCGACCGCA TTATCGCTTC TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA
8951  TGCGCAGCCT GAATGGCGAA TGGCGCCTGA TGCGGTATTT TCTCCTTACG
      ACGCGTCGGA CTTACCGCTT ACCGCGGACT ACGCCATAAA AGAGGAATGC
9001  CATCTGTGCG GTATTTCACA CCGCATATGG TGCACTCTCA GTACAATCTG
      GTAGACACGC CATAAAGTGT GGCGTATACC ACGTGAGAGT CATGTTAGAC
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9051  CTCTGATGCC GCATAGTTAA GCCAGCCCCG ACACCCGCCA ACACCCGCTG
      GAGACTACGG CGTATCAATT CGGTCGGGGC TGTGGGCGGT TGTGGGCGAC
9101  ACGCGCCCTG ACGGGCTTGT CTGCTCCCGG CATCCGCTTA CAGACAAGCT
      TGCGCGGGAC TGCCCGAACA GACGAGGGCC GTAGGCGAAT GTCTGTTCGA
9151  GTGACCGTCT CCGGGAGCTG CATGTGTCAG AGGTTTTCAC CGTCATCACC
      CACTGGCAGA GGCCCTCGAC GTACACAGTC TCCAAAAGTG GCAGTAGTGG
9201  GAAACGCGCG AGACGAAAGG GCCTCGTGAT ACGCCTATTT TTATAGGTTA
      CTTTGCGCGC TCTGCTTTCC CGGAGCACTA TGCGGATAAA AATATCCAAT
9251  ATGTCATGAT AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA
      TACAGTACTA TTATTACCAA AGAATCTGCA GTCCACCGTG AAAAGCCCCT
9301  AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT
      TTACACGCGC CTTGGGGATA AACAAATAAA AAGATTTATG TAAGTTTATA
9351  GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA
      CATAGGCGAG TACTCTGTTA TTGGGACTAT TTACGAAGTT ATTATAACTT
                                  Amp(R)
                 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9401  AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT
      TTTCCTTCTC ATACTCATAA GTTGTAAAGG CACAGCGGGA ATAAGGGAAA
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9451  TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA
      AAACGCCGTA AAACGGAAGG ACAAAAACGA GTGGGTCTTT GCGACCACTT
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9501  AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC
      TCATTTTCTA CGACTTCTAG TCAACCCACG TGCTCACCCA ATGTAGCTTG
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9551  TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT
      ACCTAGAGTT GTCGCCATTC TAGGAACTCT CAAAAGCGGG GCTTCTTGCA
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9601  TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC
      AAAGGTTACT ACTCGTGAAA ATTTCAAGAC GATACACCGC GCCATAATAG
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9651  CCGTATTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC
      GGCATAACTG CGGCCCGTTC TCGTTGAGCC AGCGGCGTAT GTGATAAGAG
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9701  AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT
      TCTTACTGAA CCAACTCATG AGTGGTCAGT GTCTTTTCGT AGAATGCCTA
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9751  GGCATGACAG TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA
      CCGTACTGTC ATTCTCTTAA TACGTCACGA CGGTATTGGT ACTCACTATT
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9801  CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA
      GTGACGCCGG TTGAATGAAG ACTGTTGCTA GCCTCCTGGC TTCCTCGATT
                            Amp(R)
      ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9851  CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG
      GGCGAAAAAA CGTGTTGTAC CCCCTAGTAC ATTGAGCGGA ACTAGCAACC
```

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                    Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9901     GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT
         CTTGGCCTCG ACTTACTTCG GTATGGTTTG CTGCTCGCAC TGTGGTGCTA
                    Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
9951     GCCTGTAGCA ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC
         CGGACATCGT TACCGTTGTT GCAACGCGTT TGATAATTGA CCGCTTGATG
                    Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10001    TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA
         AATGAGATCG AAGGGCCGTT GTTAATTATC TGACCTACCT CCGCCTATTT
                    Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10051    GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC
         CAACGTCCTG GTGAAGACGC GAGCCGGGAA GGCCGACCGA CCAAATAACG
                    Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10101    TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC
         ACTATTTAGA CCTCGGCCAC TCGCACCCAG AGCGCCATAG TAACGTCGTG
                    Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10151    TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG
         ACCCCGGTCT ACCATTCGGG AGGGCATAGC ATCAATAGAT GTGCTGCCCC
                    Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10201    AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC
         TCAGTCCGTT GATACCTACT TGCTTTATCT GTCTAGCGAC TCTATCCACG
            Amp(R)
         ~~~~~~~~~~~~~~~~~~~~~~~~
10251    CTCACTGATT AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC
         GAGTGACTAA TTCGTAACCA TTGACAGTCT GGTTCAAATG AGTATATATG
10301    TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG
         AAATCTAACT AAATTTTGAA GTAAAAATTA AATTTTCCTA GATCCACTTC
10351    ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT
         TAGGAAAAAC TATTAGAGTA CTGGTTTTAG GGAATTGCAC TCAAAAGCAA
10401    CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC
         GGTGACTCGC AGTCTGGGGC ATCTTTTCTA GTTTCCTAGA AGAACTCTAG
10451    CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA
         GAAAAAAAGA CGCGCATTAG ACGACGAACG TTTGTTTTTT TGGTGGCGAT
10501    CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA
         GGTCGCCACC AAACAAACGG CCTAGTTCTC GATGGTTGAG AAAAAGGCTT
10551    GGTAACTGGC TTCAGCAGAG CGCAGATACC AAATACTGTC CTTCTAGTGT
         CCATTGACCG AAGTCGTCTC GCGTCTATGG TTTATGACAG GAAGATCACA
10601    AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC
         TCGGCATCAA TCCGGTGGTG AAGTTCTTGA GACATCGTGG CGGATGTATG
10651    CTCGCTCTGC TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC
         GAGCGAGACG ATTAGGACAA TGGTCACCGA CGACGGTCAC CGCTATTCAG
10701    GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC
         CACAGAATGG CCCAACCTGA GTTCTGCTAT CAATGGCCTA TTCCGCGTCG
10751    GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG
         CCAGCCCGAC TTGCCCCCCA AGCACGTGTG TCGGGTCGAA CCTCGCTTGC
10801    ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC
         TGGATGTGGC TTGACTCTAT GGATGTCGCA CTCGATACTC TTTCGCGGTG
10851    GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG
         CGAAGGGCTT CCCTCTTTCC GCCTGTCCAT AGGCCATTCG CCGTCCCAGC
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10901  GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT
       CTTGTCCTCT CGCGTGCTCC CTCGAAGGTC CCCCTTTGCG GACCATAGAA
10951  TATAGTCCTG TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG
       ATATCAGGAC AGCCCAAAGC GGTGGAGACT GAACTCGCAG CTAAAAACAC
11001  ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT
       TACGAGCAGT CCCCCCGCCT CGGATACCTT TTTGCGGTCG TTGCGCCGGA
11051  TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT
       AAAATGCCAA GGACCGGAAA ACGACCGGAA AACGAGTGTA CAAGAAAGGA
11101  GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC
       CGCAATAGGG GACTAAGACA CCTATTGGCA TAATGGCGGA AACTCACTCG
11151  TGATACCGCT CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG
       ACTATGGCGA GCGGCGTCGG CTTGCTGGCT CGCGTCGCTC AGTCACTCGC
11201  AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG
       TCCTTCGCCT TCTCGCGGGT TATGCGTTTG GCGGAGAGGG GCGCGCAACC
11251  CCGATTCATT AATGCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG
       GGCTAAGTAA TTACGTCGAC CGTGCTGTCC AAAGGGCTGA CCTTTCGCCC
11301  CAGTGAGCGC AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC
       GTCACTCGCG TTGCGTTAAT TACACTCAAT CGAGTGAGTA ATCCGTGGGG
11351  AGGCTTTACA CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC
       TCCGAAATGT GAAATACGAA GGCCGAGCAT ACAACACACC TTAACACTCG
11401  GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGAATTGAA
       CCTATTGTTA AAGTGTGTCC TTTGTCGATA CTGGTACTAA TGCTTAACTT
11451  TTGCGGCCGC AATTCAACGC CGGCGTTAAG
```

FIGURE 6A

NY-ESO-1

Met Gln Ala Glu Gly Arg Gly Thr Gly Gly Ser Thr Gly Asp Ala Asp
Gly Pro Gly Gly Pro Gly Ile Pro Asp Gly Pro Gly Gly Asn Ala Gly
Gly Pro Gly Glu Ala Gly Ala Thr Gly Gly Arg Gly Pro Arg Gly Ala
Gly Ala Ala Arg Ala Ser Gly Pro Gly Gly Gly Ala Pro Arg Gly Pro
His Gly Gly Ala Ala Ser Gly Leu Asn Gly Cys Cys Arg Cys Gly Ala
Arg Gly Pro Glu Ser Arg Leu Leu Glu Phe Tyr Leu Ala Met Pro Phe
Ala Thr Pro Met Glu Ala Glu Leu Ala Arg Arg Ser Leu Ala Gln Asp
Ala Pro Pro Leu Pro Val Pro Gly Val Leu Leu Lys Glu Phe Thr Val
Ser Gly Asn Ile Leu Thr Ile Arg Leu Thr Ala Ala Asp His Arg Gln
Leu Gln Leu Ser Ile Ser Ser Cys Leu Gln Gln Leu Ser Leu Leu Met
Trp Ile Thr Gln Cys Phe Leu Pro Val Phe Leu Ala Gln Pro Pro Ser
Gly Gln Arg Arg

FIGURE 6C

TRP-2

Met Ser Pro Leu Trp Trp Gly Phe Leu Leu Ser Cys Leu Gly Cys Lys Ile
Leu Pro Gly Ala Gln Gly Gln Phe Pro Arg Val Cys Met Thr Val Asp Ser
Leu Val Asn Lys Glu Cys Cys Pro Arg Leu Gly Ala Glu Ser Ala Asn Val
Cys Gly Ser Gln Gln Gly Arg Gly Gln Cys Thr Glu Val Arg Ala Asp Thr
Arg Pro Trp Ser Gly Pro Tyr Ile Leu Arg Asn Gln Asp Asp Arg Glu Leu
Trp Pro Arg Lys Phe Phe His Arg Thr Cys Lys Cys Thr Gly Asn Phe Ala
Gly Tyr Asn Cys Gly Asp Cys Lys Phe Gly Trp Thr Gly Pro Asn Cys Glu
Arg Lys Lys Pro Pro Val Ile Arg Gln Asn Ile His Ser Leu Ser Pro Gln
Glu Arg Glu Gln Phe Leu Gly Ala Leu Asp Leu Ala Lys Lys Arg Val His
Pro Asp Tyr Val Ile Thr Thr Gln His Trp Leu Gly Leu Leu Gly Pro Asn
Gly Thr Gln Pro Gln Phe Ala Asn Cys Ser Val Tyr Asp Phe Phe Val Trp
Leu His Tyr Tyr Ser Val Arg Asp Thr Leu Leu Gly Pro Gly Arg Pro Tyr
Arg Ala Ile Asp Phe Ser His Gln Gly Pro Ala Phe Val Thr Trp His Arg
Tyr His Leu Leu Cys Leu Glu Arg Asp Leu Gln Arg Leu Ile Gly Asn Glu
Ser Phe Ala Leu Pro Tyr Trp Asn Phe Ala Thr Gly Arg Asn Glu Cys Asp
Val Cys Thr Asp Gln Leu Phe Gly Ala Ala Arg Pro Asp Asp Pro Thr Leu
Ile Ser Arg Asn Ser Arg Phe Ser Ser Trp Glu Thr Val Cys Asp Ser Leu
Asp Asp Tyr Asn His Leu Val Thr Leu Cys Asn Gly Thr Tyr Glu Gly Leu
Leu Arg Arg Asn Gln Met Gly Arg Asn Ser Met Lys Leu Pro Thr Leu Lys
Asp Ile Arg Asp Cys Leu Ser Leu Gln Lys Phe Asp Asn Pro Pro Phe Phe
Gln Asn Ser Thr Phe Ser Phe Arg Asn Ala Leu Glu Gly Phe Asp Lys Ala
Asp Gly Thr Leu Asp Ser Gln Val Met Ser Leu His Asn Leu Val His Ser
Phe Leu Asn Gly Thr Asn Ala Leu Pro His Ser Ala Ala Asn Asp Pro Ile
Phe Val Val Leu His Ser Phe Thr Asp Ala Ile Phe Asp Glu Trp Met Lys
Arg Phe Asn Pro Pro Ala Asp Ala Trp Pro Gln Glu Leu Ala Pro Ile Gly
His Asn Arg Met Tyr Asn Met Val Pro Phe Phe Pro Pro Val Thr Asn Glu
Glu Leu Phe Leu Thr Ser Asp Gln Leu Gly Tyr Ser Tyr Ala Ile Asp Leu
Pro Val Ser Val Glu Glu Thr Pro Gly Trp Pro Thr Thr Leu Leu Val Val
Met Gly Thr Leu Val Ala Leu Val Gly Leu Phe Val Leu Leu Ala Phe Leu
Gln Tyr Arg Arg Leu Arg Lys Gly Tyr Thr Pro Leu Met Glu Thr His Leu
Ser Ser Lys Arg Tyr Thr Glu Glu Ala

FIGURE 6D

gp100 and gp100M

```
1        MDL VLKRCLLHLA VIGALLAVGA TKVPRNQDWL GVSRQLRTKA WNRQLYPEWT
2        *** ********** ********** ********** ********** **********

1 EAQRLDCWRG GQVSLKVSND GPTLIGANAS FSIALNFPGS QKVLPDGQVI WVNNTIINGS
2 ********** ********** ********** ********** ********** **********

1 QVWGGQPVYP QETDDACIFP DGGPCPSGSW SQKRSFVYVW KTWGQYWQFL GGPVSGLSIG
2 ********** ********** ********** ********** ********V* **********

1 TGRAMLGTHT MEVTVYHRRG SRSYVPLAHS SSAFTITDQV PFSVSVSQLR ALDGGNKHFL
2 ********** ********** ********** ******M*** ********** **********

1 RNQPLTFALQ LHDPSGYLAE ADLSYTWDFG DSSGTLISRA LVVTHTYLEP GPVTAQVVLQ
2 ********** ********** ********** ********** ********** ****V*****

1 AAIPLTSCGS SPVPGTTDGH RPTAEAPNTT AGQVPTTEVV GTTPGQAPTA EPSGTTSVQV
2 ********** ********** ********** ********** ********** **********

1 PTTEVISTAP VQMPTAESTG MTPEKVPVSE VMGTTLAEMS TPEATGMTPA EVSIVVLSGT
2 ********** ********** ********** ********** ********** **********

1 TAAQVTTTEW VETTARELPI PEPEGPDASS IMSTESITGS LGPLLDGTAT LRLVKRQVPL
2 ********** ********** ********** ********** ********** **********

1 DCVLYRYGSF SVTLDIVQGI ESAEILQAVP SGEGDAFELT VSCQGGLPKE ACMEISSPGC
2 ********** ********** ********** ********** ********** **********

1 QPPAQRLCQP VLPSPACQLV LHQILKGGSG TYCLNVSLAD TNSLAVVSTQ LIMPGQEAGL
2 ********** ********** ********** ********** ********** **********

1 GQVPLIVGIL LVLMAVVLAS LIYRRRLMKQ DFSVPQLPHS SSHWLRLPRI FCSCPIGENS
2 ********** ********** ********** ********** ********** **********

1 PLLSGQQV2 ********
```

Key
*=identical amino acid residue
1=gp100
2=gp100M

FIGURE 6E

MART-1

Met Pro Arg Glu Asp Ala His Phe Ile Tyr Gly Tyr Pro Lys Lys Gly His Gly His Ser Tyr Thr Thr Ala Glu Glu Ala Ala Gly Ile Gly Ile Leu Thr Val Ile Leu Gly Val Leu Leu Leu Ile Gly Cys Trp Tyr Cys Arg Arg Arg Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val Gly Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp His Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val Val Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser Pro Pro Pro Tyr Ser Pro

FIGURE 6F

MAGE-1

Met Ser Asp Asn Lys Lys Pro Asp Lys Ala His Ser Gly Ser Gly Gly
Asp Gly Asp Gly Asn Arg Cys Asn Leu Leu His Arg Tyr Ser Leu Glu
Glu Ile Leu Pro Tyr Leu Gly Trp Leu Val Phe Ala Val Val Thr Thr
Ser Phe Leu Ala Leu Gln Met Phe Ile Asp Ala Leu Tyr Glu Glu Gln
Tyr Glu Arg Asp Val Ala Trp Ile Ala Arg Gln Ser Lys Arg Met Ser
Ser Val Asp Glu Asp Glu Asp Asp Glu Asp Asp Glu Asp Asp Tyr Tyr
Asp Asp Glu Asp Asp Asp Asp Asp Ala Phe Tyr Asp Asp Glu Asp Asp
Glu Glu Glu Glu Leu Glu Asn Leu Met Asp Asp Glu Ser Glu Asp Glu
Ala Glu Glu Glu Met Ser Val Glu Met Gly Ala Gly Ala Glu Glu Met
Gly Ala Gly Ala Asn Cys Ala Cys Val Pro Gly His His Leu Arg Lys
Asn Glu Val Lys Cys Arg Met Ile Tyr Phe Phe His Asp Pro Asn Phe
Leu Val Ser Ile Pro Val Asn Pro Lys Glu Gln Met Glu Cys Arg Cys
Glu Asn Ala Asp Glu Glu Val Ala Met Glu Glu Glu Glu Glu Glu Glu
Glu Glu Glu Glu Glu Glu Glu Met Gly Asn Pro Asp Gly Phe Ser Pro

FIGURE 6G

MAGE-3

mpleqrsqhc kpeeglearg ealglvgaqa pateeqeaas ssstlvevtl gevpaaespd
ppqspqgass lpttmnyplw sqsyedssnq eeegpstfpd lesefqaals rkvaelvhfl
llkyararepv tkaemlgsvv gnwqyffpvi fskassslql vfgielmevd pighlyifat
clglsydgll gdnqimpkag lliivlaiia regdcapeek iweelsvlev fegredsilg
dpkklltqhf vqenyleyrq vpgsdpacye flwgpralve tsyvkvlhhm vkisggphis
ypplhewvlr egee

FIGURE 6H
B7.1

mghtrrqgts pskcpylnff qllvlaglsh fcsgvihvtk evkevatlsc ghnvsveela
qtriywqkek kmvltmmsgd mniwpeyknr tifditnnls ivilalrpsd egtyecvvlk
yekdafkreh laevtlsvka dfptpsisdf eiptsnirri icstsggfpe phlswlenge
elnainttvs qdpetelyav sskldfnmtt nhsfmcliky ghlrvnqtfn wnttkqehfp
dnllpswait lisvngifvi ccltycfapr crerrrnerl rresvrpv

FIGURE 6I
LFA-3

mvagsdagra lgvlsvvcll hcfgfiscfs qqiygvvygn vtfhvpsnvp lkevlwkkqk
dkvaelense frafssfknr vyldtvsgsl tiynltssde deyemespni tdtmkfflyv
leslpsptlt caltngsiev qcmipehyns hrglimyswd cpmeqckrns tsiyfkmend
lpqkiqctls nplfnttssi ilttcipssg hsrhryalip iplavittci vlymngilkc
drkpdrtnsn

FIGURE 6J
ICAM-1*

mapssprpal pallvllgal fpgpgnaqts vspskvilpr ggsvlvtcst scdqpkllgi
etplpkkell lpgnnrkvye lsnvqedsqp mcysncpdgq staktfltvy wtpervelap
lpswqpvgkn ltlrcqvegg apranltvvl lrgekelkre pavgepaevt ttvlvrrdhh
ganfscrtel dlrpqglelf entsapyqlq tfvlpatppq lvsprvlevd tqgtvvcsld
glfpvseaqv hlalgdqrln ptvtygndsf sakasvsvta edegtqrltc avilgnqsqe
tlqtvtiysf papnviltkp evsegtevtv kceahprakv tlngvpaqpl gpraqlllka
tpedngrsfs csatlevagq lihknqtrel rvlygprlde rdcpgnwtwp ensqqtpmcq
awgnplpelk clkdgtfplp igesvtvtrd legtylcrar stqgevtrev tvnvlsprye
iviitvvaaa vimgtaglst ylynrqrkik kyrlqqaqkg tpmkpntqat pp

*mature sequence begins at residue 28 (q)